THE SEGREGATION OF CARBOHYDRATES IN CROSSES BETWEEN WAXY AND STARCHY TYPES OF MAIZE[1]

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The purpose of this paper is to report the Mendelian segregation of waxy and starchy carbohydrates in both the sporophytic and gametophytic generations of maize. This is of especial genetic interest because the counts of segregate waxy kernels have very commonly been short of the expected numbers, although this character appears to be a simple Mendelian recessive. COLLINS (1909) was the first to point out the physical difference in the endosperm of waxy and ordinary starchy varieties.

While the exact difference in their chemical nature has not been established, WEATHERWAX (1922) considered that the waxy carbohydrate was erythrodextrin because of its reddish color reaction to iodine as compared with the bluish staining of the endosperms of ordinary starchy varieties. DEMEREC (1924), BRINK AND MACGILLIVRAY (1924), AND LONGLEY (1924) have each established a corresponding differential staining of the pollen grains of the two maize sorts. BRINK (1925) has more recently reported finding the same chemical distinction for the carbohydrates of the embryo sacs. In a publication of earlier results the writers (1925) failed, through faulty technique, to observe the differential staining of the pollen, but later results conform with those of the above investigators. Our earlier

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observations as to the wide distribution of starch throughout the sporophytic vegetative tissues have been confirmed.

Taking into consideration the observations of all investigators, it appears that the waxy carbohydrate is restricted to haploid or 1x tissue including pollen, embryo sac, and possibly the tissue developed from the antipodals, and to the 3x tissue of the endosperm. Only ordinary starch has been found in the diploid or 2x tissues which make up the rest of the plant.

The results of this investigation as well as those of DEMEREC, BRINK AND MACGILLIVRAY, AND LONGLEY indicate that those segregated plants following hybridization which come true to endosperm type also come true to the same sort of carbohydrate in the pollen. This is evidence that a single chromosome factor controls both or else that they are very closely linked characters with no observed crossing over.

DISTINGUISHING WAXY AND STARCHY CARBOHYDRATES

Pollen

Repeated attempts to distinguish the pollen of Chinese waxy maize and its hybrids from ordinary starchy maize pollen by the iodine test during the summer of 1924, following receipt of the articles by DEMEREC, and BRINK and MACGILLIVRAY, resulted in failure so that we concluded that such a difference did not exist. Further tests during 1925 showed that with proper staining it is easy to distinguish the two types of pollen and show the segregation in hybrids.

The following method was used succesfully: The pollen was placed on a slide with a few drops of stock solution of iodine in potassium iodide[1] diluted with 3 parts of distilled water. The slide was then left uncovered, more iodine solution being added if necessary, until the pollen appeared sufficiently stained. A small crystal of chloral hydrate was then added as a clearing agent. When this was dissolved the cover glass was applied. The pollen should all be moistened at the same time in order to stain each type of pollen uniformly.

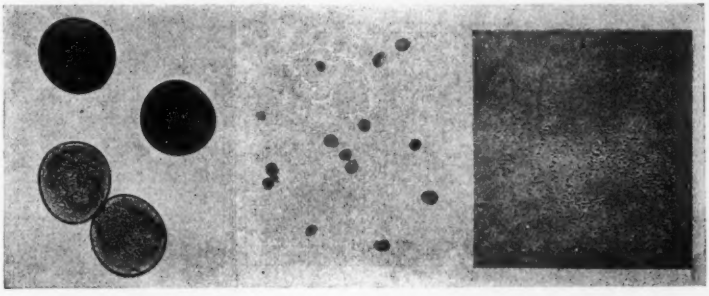
By this method the carbohydrate grains of the pollen of ordinary starchy maize are stained a deep blue, while those in waxy maize are stained red, leaving other parts of the pollen grains unstained and cleared. When pollen is stained in this manner, any imperfect pollen grains which lack carbohydrate reserves remain unstained and may be distinguished from the normal pollen. These methods apply to pollen stored dry as well as in the fresh condition.

[1] The stock solution was made by dissolving 1 gram of potassium iodide and 0.3 gram iodine in 100 cc. distilled water.

Endosperm

Satisfactory differential staining of the carbohydrates in the endosperm is more easily secured than in the case of the pollen, due, perhaps, to the much larger starch grains in the endosperm. (Figures 1b and 1c). If too strong iodine solution is used, there may be a tendency to produce such an intense stain that the endosperms all appear black rather than red or blue. The ordinary starchy endosperm seems to have a greater affinity for the iodine and will stain in a weaker solution than the waxy.

For extensive observations, rapid results may be secured by dipping the ears into iodine solution after the endosperms have been exposed by cut-



a b c

Figure 1a. Representative pollen grains from a heterozygous plant showing segregation into equal numbers of waxy and starchy grains which stain red and blue respectively, with iodine. Those which stain red and carry the factor for waxy are shown light in this photograph.

Figure 1b. Typical carbohydrate grains from the endosperm of maize, to be compared for size with those from pollen, shown in figure 1c.

Figure 1c. Typical carbohydrate grains from pollen to be compared for size with those from the endosperm of maize, shown in figure 1b.

ting off the crowns from the kernels. This may be done easily before the grain has hardened. The ears stain more promptly and evenly when first dipped momentarily in boiling water. After staining, the ears may be rinsed with tap water to remove loose starch which might cause confusion. Should the color fade before completing the counts, the ear may be restained.

Prior to the discovery of the difference in staining with iodine in 1922, all separations of starchy and waxy kernels were necessarily based on their difference in physical appearance. This can be done fairly accurately when maize is thoroughly cured. A dark-colored aleurone layer which is

frequently present in crosses with Chinese maize tends to obscure the difference and makes such separation more difficult. If the starchy segregates are of a "soft flour" type, the distinction is far less certain than in the case of more horny texture.

TABLE 1

Pollen segregation in hybrids of waxy×starchy maize, as determined by the reaction to iodine.

Tassel no.	Number staining			Ratio red to blue	Tassel no.	Number staining			Ratio red to blue
	Blue	Red	No stain			Blue	Red	No stain	
					F_1 Reid Yellow Dent×Chinese				
1	125	124	2	1.00	6	193	152	3	0.79
2	94	92	..	0.99	7	118	112	..	0.95
3	131	156	5	1.19	8	210	217	2	1.03
4	102	137	2	1.34	9	289	271	1	0.95
5	175	189	4	1.08	10	294	314	..	1.06
					Total	1731	1764	19	1.02
					F_1 Hogue Yellow Dent×Chinese				
11	314	354	2	1.13	14	302	296	4	0.93
12	356	335	7	0.94	15	324	358	2	1.10
13	355	369	3	1.04	16	334	296	1	0.89
					Total	1985	2008	19	1.01
					F_2 Chinese×Yellow Flint				
17	295	292	..	0.99	37	167	184	6	1.10
18	128	157	..	1.23	38	308	310	8	1.01
19	340	284	..	0.84	39	341	346	2	1.01
20	321	279	..	0.87	40	73	94	..	1.29
21	190	198	..	1.04	41	264	269	..	1.02
22	424	493	..	1.16	42	109	109	..	1.00
23	191	180	6	0.94	43	186	170	..	0.91
24	139	122	5	0.88	44	143	129	5	0.90
25	336	315	2	0.94	45	75	57	..	0.76
26	225	229	8	1.02	46	56	55	..	0.98
27	242	327	21	1.35	47	196	215	6	1.10
28	201	252	..	1.25	48	267	323	1	1.21
29	424	428	..	1.01	49	299	301	9	1.01
30	207	191	7	0.92	50	748	734	..	0.98
31	112	116	..	1.04	51	236	257	..	1.09
32	201	206	..	1.02	52	296	308	..	1.04
33	35	43	63	1.23	53	384	378	14	0.98
34	28	25	..	0.89	54	290	261	3	0.90
35	347	392	21	1.13	55	41	45	2	1.10
36	118	85	36	0.72	56	315	298	..	0.95
					Total	9298	9457	225	1.02

SEGREGATION OF POLLEN

Counts were made for the pollen from 88 segregating plants, resulting in 13014 pollen grains stained blue and 13229 stained red. This is 49.59 percent blue and 50.41 percent red. The imperfect grains which often appear have not been included in calculating the ratios. The results are given in table 1. The variations from the 1:1 ratio are doubtless due mainly to the use of samples which were not exactly representative rather than to actual differences in number of the two types of pollen. This statement is confirmed by the lack of correlation between pollen and kernel ratios as shown in table 2.

DEMEREC (1924), and BRINK and MACGILLIVRAY (1924) reported that approximately equal numbers of the two types of pollen grains were found in plants heterozygous for the waxy character. LONGLEY (1924) has also reported such segregation in the pollen of crosses of waxy with starchy maize and teosinte, and in crosses between starchy and waxy Coix. PARNELL had previously reported segregation of the pollen in crosses between glutinous and ordinary rice.

SEGREGATION OF ENDOSPERM

A large number of ears were hand-pollinated with segregating pollen. These belong to 3 groups: (1) pure Chinese ears pollinated with F_1 waxy $\times$ starchy pollen; (2) F_1 waxy $\times$ starchy plants selfed; and (3) F_2 plants grown from starchy segregated seed, selfed. In the pure Chinese fertilized by F_1 pollen 50 percent waxy kernels would be expected while in the case of F_1 and F_2 ears, the expectation would be 25 percent waxy kernels.

The ear shoots were bagged sufficiently in advance of silking to insure against contamination by stray viable pollen. As an additional precaution the pure Chinese maize was grown in isolation and detasseled because this variety tends to silk while the shoots are small and still enclosed within the leaf sheath.

Segregation where only 1 parent is heterozygous.

In this test pure waxy maize was fertilized by pollen from F_1 starchy $\times$ waxy plants. Since the ovules all carried the recessive waxy factor, the ratio of the two types of pollen would determine the kernel segregation unless influenced by differential fertilization.

Sixty-six ears of this combination were obtained as reported in table 2. These ears had been fertilized by pollen from 9 F_1 Reid $\times$ Chinese plants whose pollen segregation had been established by the iodine stain. Of

18549 kernels produced 51.5 percent proved to be waxy as determined mainly by the iodine test.

TABLE 2

Endosperm segregation of pure waxy (Chinese) maize fertilized by segregating pollen from F_1, waxy×starchy (Reid×Chinese) plants.

Ear No.	Number of kernels			Percent waxy kernels	Percent deviation of waxy	Dev.	Pollen	
	Total	Waxy	Expected waxy			P.E.†	From tassel No. (Table 1)	Percent staining red*
1	343	180	171	53.5	3.5	1.44	1	49.8
2	386	186	193	48.2	−1.8	1.06	1	49.8
3	291	152	146	52.2	2.2	1.04	2	49.5
4	209	115	104	55.0	5.0	2.26	2	49.5
5	301	166	151	55.1	5.1	2.56	2	49.5
6	143	74	71	51.8	1.8	0.74	2	49.5
7	55	21	28	38.2	−11.8	3.11	2	49.5
8	127	69	63	54.3	4.3	1.58	2	49.5
9	222	98	111	44.1	−5.9	2.59	3	54.4
10	432	236	216	54.6	4.6	2.85	3	54.4
11	410	229	205	55.9	5.9	3.51	3	54.4
12	325	151	163	46.5	−3.5	1.97	3	54.4
13	398	229	199	57.5	7.5	4.46	3	54.4
14	348	182	174	52.3	2.3	1.27	3	54.4
15	306	171	153	55.9	5.9	3.05	4	57.3
16	120	67	60	55.8	5.8	1.90	4	57.3
17	273	121	137	44.3	−5.7	2.87	4	57.3
18	345	155	172	44.9	−5.1	2.72	4	57.3
19	420	223	210	53.1	3.1	1.88	4	57.3
20	389	175	195	45.0	−5.0	3.01	4	57.3
21	427	249	213	58.3	8.3	5.16	4	57.3
22	193	98	97	50.8	0.8	....	4	57.3
23	95	52	47	54.7	4.7	1.52	5	51.9
24	317	159	158	50.2	0.2	0.16	5	51.9
25	105	52	53	49.5	−0.5	0.29	5	51.9
26	160	73	80	45.6	−4.4	1.64	5	51.9
27	94	50	47	53.2	3.2	0.92	5	51.9
28	105	56	52	53.3	3.3	1.16	5	51.9
29	276	151	138	54.7	4.7	2.32	5	51.9
30	163	96	82	58.9	8.9	3.25	5	51.9
31	459	241	229	52.5	2.5	1.64	5	51.9
32	332	162	166	48.8	−1.2	0.65	5	51.9
33	272	160	136	58.8	8.8	4.32	5	51.9
34	241	130	121	54.5	4.5	1.71	5	51.9
35	254	143	127	56.3	6.3	2.98	5	51.9
36	346	188	173	54.3	4.3	2.39	5	51.9
37	323	199	162	61.6	11.6	4.46	5	51.9
38	366	182	183	49.7	−0.3	0.15	5	51.9
39	401	192	201	47.9	−2.1	1.33	5	51.9
40	456	196	228	43.0	−7.0	4.44	5	51.9
41	448	208	224	46.4	−3.6	2.24	5	51.9
42	339	165	170	48.7	−1.3	0.81	6	44.1

TABLE 2 (continued)

Endosperm segregation of pure waxy (Chinese) maize fertilized by segregating pollen from F_1, waxy×starchy (Reid×Chinese) plants.

Ear No.	Number of kernels			Percent waxy kernels	Percent deviation of waxy	Dev. / P.E.†	Pollen	
	Total	Waxy	Expected waxy				From tassel No. (Table 1)	Percent staining red*
43	348	184	174	52.9	2.9	1.59	6	44.1
44	286	133	143	46.5	−3.5	1.75	6	44.1
45	357	166	178	46.5	−3.5	1.88	6	44.1
46	360	171	180	47.5	−2.5	1.41	6	44.1
47	251	133	125	53.0	3.0	1.27	7	48.7
48	305	142	153	46.6	−3.4	1.87	7	48.7
49	335	172	167	51.3	1.3	0.81	7	48.7
50	428	193	214	45.1	−4.9	3.01	7	48.7
51	190	107	95	56.3	6.3	2.58	9	48.4
52	261	145	131	55.6	5.6	2.57	9	48.4
53	205	113	102	55.1	5.1	2.28	9	48.4
54	412	214	206	51.9	1.9	1.17	9	48.4
55	325	164	163	50.5	0.5	0.16	9	48.4
56	425	250	212	58.8	8.8	5.47	9	48.4
57	273	143	137	52.4	2.4	1.08	10	51.6
58	65	37	32	56.9	6.9	1.84	10	51.6
59	78	43	39	55.1	5.1	1.34	10	51.6
60	232	126	116	54.3	4.3	1.95	10	51.6
61	215	113	108	52.6	2.6	1.01	10	51.6
62	81	39	40	48.2	−1.8	3.29	10	51.6
63	243	130	122	53.5	3.5	1.52	10	51.6
64	171	86	85	50.3	0.3	2.27	10	51.6
65	342	175	171	51.2	1.2	0.64	10	51.6
66	346	166	173	48.0	−2.0	1.11	10	51.6
Total	18549	9547	9275	51.5	1.5	5.92		
Ears grouped according to source of pollen								
1–2	729	368	365	50.5	0.5	0.33	1	49.8
3–8	1126	597	563	53.0	3.0	3.00	2	49.5
9–14	2135	1125	1068	52.7	2.7	3.66	3	54.4
15–22	2473	1259	1236	50.9	0.9	1.37	4	57.3
23–41	5213	2690	2606	51.6	1.6	2.45	5	51.9
42–46	1690	819	845	48.5	−1.5	1.88	6	44.4
47–50	1319	640	660	48.5	−1.5	1.63	7	48.7
51–56	1818	993	909	54.6	4.6	5.85	9	48.4
57–66	2046	1058	1023	51.7	1.7	2.30	10	51.6
1–66	18549	9547	9275	51.5	1.5	5.92		50.5

†Probable errors were calculated by the usual formula: $.6745\sqrt{P \times Q} \times \sqrt{\text{Total number}}$

*Imperfect pollen grains which failed to stain were not included in the percentage calculation.

In comparison with these results (table 3) in which the male parent was segregating, KEMPTON (1919) found an average shortage of 0.8 percent for the waxy kernels although one combination involving 8791 kernels showed no deficiency. Data reported by BREGGER (1918) in connection with linkage studies show a one-percent shortage from the expected number of waxy kernels. With 33020 kernels, BRINK (1925) shows a deficiency of 1.7 percent. Summarizing all of the results, there are 203 ears with 79,381 kernels of which 49.3 percent are waxy.

TABLE 3

Historical summary showing the endosperm segregation in pure waxy maize fertilized by segregating pollen from waxy×starchy hybrids. The expected ratio is 50 percent waxy kernels.

Investigators and their table numbers	Number of ears	Number of kernels			Percent waxy	Percent deviation of waxy	Dev. / P.E.	Percent of ears below 50% waxy
		Total	Waxy	Expected waxy				
KEMPTON (1919) Table 3	27	11,724	5,802	5,862	49.6	−0.4	1.65	56
KEMPTON (1919) Table 6	20	8,791	4,397	4,396	50.0	0.0	0.00	45
KEMPTON (1919) Table 10	11	6,138	2,925	3,069	47.7	−2.3	5.44	73
Total—KEMPTON	58	26,653	13,124	13,327	49.2	−0.8	3.69	55
BREGGER (1918)	4	1,159	568	580	49.0	−1.0	1.05	50
BRINK (1925) Tables 15–16	75	33,020	15,934	16,510	48.3	−1.7	9.38	67
KIESSELBACH AND PETERSEN (1926) Table 2	66	18,549	9,547	9,275	51.5	1.5	5.92	33
Total	203	79,381	39,173	39,690	49.3	−0.7	5.44	52

In reverse combinations in which the female was the segregating parent, 5 investigators (table 4) report segregations which total a deficiency of 0.02 percent from the expected percentage of waxy kernels. These tests represent a total of 198 ears and 90,944 kernels.

Segregation where both parents are heterozygous.

In these tests both F_1 and F_2 hybrids were included. Since half of the ovules carry the dominant starchy factor, the kernels which result from them will be starchy regardless of the type of pollen. The ratio of the waxy and starchy kernels produced from the other half will be determined by

the ratio of the 2 types of functioning pollen grains. Thus approximately a 3:1 ratio is expected in these combinations. The ears of two varietal F_1 crosses, Reid × Chinese and Chinese × Hogue were selfed for an investigation of kernel segregation. A total of 39 selfed ears were obtained, 23 of the former and 16 of the latter cross (table 5). Of a total of 12,376 kernels in the Reid × Chinese cross, 23.2 percent were waxy, while 25.1 percent of the 8,908 Chinese × Hogue kernels were waxy. Twenty-four

TABLE 4

Historical summary showing the endosperm segregation in heterozygous ears from waxy × starchy hybrids fertilized by pollen from pure waxy maize. The expected ratio is 50 percent waxy kernels.

Investigators and their table numbers	Number of ears	Number of kernels			Percent waxy	Percent deviation of waxy	Dev. / P.E.	Percent of ears below 50% waxy
		Total	Waxy	Expected waxy				
Collins and Kempton (1911) Table 2	2	1,228	526	614	42.8	−7.2	7.5	100
Kempton (1919) Table 4	39	15,305	7,817	7,653	51.0	1.0	3.93	31
Kempton (1919) Table 7	26	13,027	6,447	6,514	49.5	−0.5	1.71	62
Kempton (1919) Table 11	8	2,866	1,451	1,433	50.7	0.7	1.00	38
Total—Collins and Kempton, and Kempton	75	32,426	16,241	16,213	50.1	0.1	..	80
Hutchison Tables 4, 5, 6, and 7	35	19,032	9,583	9,516	50.4	1.4	1.55	46
Bregger (1918)	6	2,437	1,173	1,219	48.1	−1.9	2.76	67
Brink (1925) Tables 13, 14	82	37,049	18,457	18,525	49.8	−0.2	1.05	56
Total	198	90,944	45,454	45,472	49.98	−0.02	0.18	50

percent of all the kernels from both combinations were waxy. In another test starchy segregate seed from selfed F_1 ears of a Chinese × Flint cross were planted for a further study of segregation in the F_2 generation. Of the 101 selfed ears produced, 68 were found to be segregating. This corresponds almost exactly with the expected 2:1 ratio. Of the 19,126 kernels (table 6) produced on these segregating ears, 24.2 percent were of the waxy type.

TABLE 5

Endosperm segregation in selfed F_1 waxy×starchy maize hybrids.

Ear No.	Number of kernels			Percent waxy	Percent deviation of waxy	Dev. / P.E.	Pollen from tassel no. (Table 1)
	Total	Waxy	Expected waxy				
			Reid Yellow Dent×Chinese				
1	502	141	126	28.1	3.1	2.29	*
2	609	137	152	22.5	−2.5	2.08	*
3	765	170	191	22.2	−2.8	2.60	*
4	726	177	182	24.4	−0.6	0.64	*
5	638	146	159	22.9	−2.1	1.76	*
6	802	181	201	22.6	−2.4	2.42	1
7	734	172	183	23.4	−1.6	1.39	2
8	22	3	5	13.6	−11.4	1.47	3
9	490	112	122	22.9	−2.1	1.55	*
10	571	133	143	23.3	−1.7	1.43	4
11	879	235	220	26.7	1.7	1.73	5
12	240	51	60	21.3	−3.7	1.99	7
13	140	42	35	30.0	5.0	2.03	8
14	597	143	149	24.0	−1.0	0.84	9
15	304	78	76	25.7	0.7	0.39	*
16	263	68	66	25.9	0.9	0.42	*
17†	762	172	190	22.6	−2.4	2.23	10
18	337	75	84	22.3	−2.7	1.68	*
19	464	84	116	18.1	−6.9	5.09	*
20	671	135	168	20.1	−4.9	4.37	*
21	695	157	174	22.6	−2.4	2.21	*
22	602	131	151	21.8	−3.2	2.79	*
23	563	124	141	22.0	−3.0	2.45	*
Total	12376	2868	3094	23.2	−1.8	6.93	
			Chinese×Hogue Yellow Dent				
24	592	147	148	24.8	−0.2	0.14	*
25	596	140	149	23.5	−1.5	1.26	*
26	575	136	144	23.7	−1.3	1.14	*
27	583	152	146	26.2	1.2	0.85	*
28	668	180	167	26.9	1.9	1.72	*
29	789	212	197	26.9	1.9	1.83	*
30	191	47	48	24.6	−0.4	0.25	11
31	579	165	145	28.5	3.5	2.84	*
32	428	102	107	23.8	−1.2	0.83	12
33	646	180	162	27.9	2.9	2.43	*
34	362	113	91	31.2	6.2	3.96	*
35	86	49	72	17.1	−7.9	4.65	*
36	605	136	151	22.5	−2.5	2.09	*
37	808	195	202	24.1	−0.9	0.98	*
38	637	140	159	22.0	−3.0	2.59	*
39	563	139	141	24.7	−0.1	0.29	*
Total	8908	2233	2227	25.1	0.1	0.22	
Total of both crosses	21284	5101	5321	24.0	−1.0	5.14	

*Where tassel numbers are omitted the pollen had not been examined.

†The bracket indicates that the two ears were borne on the same plant.

TABLE 6

Endosperm segregation in selfed F_2 plants heterozygous for the waxy character in a waxy×starchy (Chinese×Yellow Flint) hybrid.

Ear No.	Number of kernels			Percent waxy kernels	Percent deviation of waxy	Dev. / P.E.	Pollen from tassel No. (Table 7)
	Total	Waxy	Expected waxy				
1	312	60	78	19.2	−5.8	3.49	*
2†	295	67	74	22.7	−2.3	1.39	14
3	288	63	72	21.9	−3.1	1.83	14
4	328	89	82	27.4	2.4	1.32	18
5	313	78	78	24.9	−0.1	0.19	*
6	296	79	74	26.7	1.7	1.00	19
7	127	31	32	24.4	−0.6	0.30	*
8	120	24	30	20.0	−5.0	1.86	*
9	224	56	56	25.0	0.0	....	22
10	328	82	82	25.0	0.0	....	*
11	270	56	68	20.7	−4.3	2.52	24
12	251	66	63	26.3	1.3	0.65	24
13	417	89	104	21.3	−3.7	2.68	25
14	118	30	30	25.4	0.4	....	26
15	305	80	76	26.2	1.2	0.78	27
16	158	35	40	22.2	−2.8	1.63	28
17	346	80	86	23.1	−1.9	1.10	29
18	209	58	52	27.8	2.8	1.42	32
19	151	39	38	25.8	0.8	0.28	33
20	463	128	116	27.7	2.7	1.91	34
21	303	79	76	26.1	1.1	0.59	35
22	301	72	75	23.9	−1.1	0.59	37
23	379	105	95	27.7	2.7	1.76	38
24	347	84	87	24.2	−0.8	0.55	39
25	126	26	32	20.6	−4.4	1.83	42
26	74	19	18	25.7	0.7	0.40	47
27	202	56	51	27.7	2.7	1.20	50
28	80	19	20	23.8	−1.2	0.38	55
29	393	99	98	25.2	0.2	1.72	*
30	193	49	48	25.4	0.4	0.25	*
31	345	83	86	24.1	−0.9	0.55	*
32	219	49	55	22.4	−2.6	1.39	*
33	367	98	92	26.7	1.7	1.07	*
34	243	52	61	21.4	−3.6	1.98	*
35	377	89	94	23.6	−1.4	1.06	*
36	200	49	50	24.3	−0.7	0.24	*
37	337	93	84	27.6	2.6	1.68	*
38	219	58	55	26.5	1.5	0.69	*
39	66	25	17	37.9	12.9	3.38	*
40	337	82	84	24.3	−0.7	0.37	*
41	357	87	89	24.4	−0.6	0.36	*
42	117	35	29	29.9	4.9	1.90	*

*Where tassel numbers are omitted the pollen had not been examined.

†The bracket indicates that the two ears were borne on the same plant.

TABLE 6 (continued)

Endosperm segregation in selfed F_2 plants heterozygous for the waxy character in a waxy×starchy (Chinese×Yellow Flint) hybrid.

EAR NO.	NUMBER OF KERNELS			PERCENT WAXY KERNELS	PERCENT DEVIATION OF WAXY	DEV. / P.E.	POLLEN FROM TASSEL NO. (Table 7)
	Total	Waxy	Expected waxy				
43	421	97	105	23.0	−2.0	1.82	44
44	312	72	78	23.1	−1.9	1.16	48
45	400	108	100	27.0	2.0	1.37	*
46	188	48	47	25.5	0.5	0.25	*
47	236	48	59	20.3	−4.7	2.84	*
48	357	89	89	24.9	−0.1	..	*
49	354	92	89	26.0	1.0	0.55	*
50	292	69	73	23.6	−1.4	1.60	*
†51	342	66	85	19.3	−5.7	4.26	*
†52	315	60	79	19.0	−6.0	3.67	*
†53	479	114	120	23.8	−1.2	1.25	*
†54	370	77	92	20.8	−4.2	3.38	*
55	206	38	52	18.4	−6.6	3.34	*
56	172	38	43	22.1	−2.9	0.31	*
57	214	46	53	21.5	−3.5	1.64	*
58	44	12	11	27.3	2.3	0.52	51
59	249	52	62	20.9	−4.1	2.17	*
60	349	86	87	24.6	−0.4	0.37	*
61	389	72	97	18.5	−6.5	4.34	*
62	475	115	119	24.2	−0.8	0.63	*
63	362	94	90	26.0	1.0	0.72	*
64	538	129	135	24.0	−1.0	0.98	*
65	381	130	95	34.1	9.1	6.14	*
66	264	72	66	27.3	2.3	1.26	*
67	208	34	52	16.4	−8.6	4.28	*
68	308	67	77	21.8	−3.2	1.95	*
Total	19126	4623	4781	24.2	−0.8	3.91	

*Where tassel numbers are omitted, the pollen had not been examined.
†The bracket indicates that the two ears were borne on the same plant.

A summary of the results (table 7) obtained by COLLINS and KEMPTON (1911), and KEMPTON (1919) from crosses corresponding with the above in that both parents were heterozygous show that of 102,429 kernels 23.9 percent were waxy. In observations by BRINK (1925) with 10,032 kernels, 22.9 percent were of the waxy type. Summarizing all data where both parents were heterozygous, 356 ears including 152,871 kernels resulted in 23.9 percent waxy kernels.

TABLE 7

Historical summary showing the endosperm segregation where both parents are heterozygous for the waxy character. The expected ratio is 25 percent waxy kernels

INVESTIGATORS AND THEIR TABLE NUMBERS	No. OF EARS	NUMBER OF KERNELS			PER-CENT WAXY	PERCENT DEVIATION OF WAXY	DEV. / P.E.	PERCENT OF EARS BELOW 25 % WAXY
		Total	Waxy	Expected waxy				
COLLINS and KEMPTON (1911) Table 1	45	22,339	5,179	5,585	23.1	−1.9	9.24	64
KEMPTON (1919) Table 1	96	54,759	13,088	13,690	23.9	−1.1	8.8	65
KEMPTON (1919) Table 4	28	12,474	3,114	3,118	25.0	0.0	0.1	54
KEMPTON (1919) Table 7	17	8,177	1,981	2,044	24.3	−0.7	2.4	71
KEMPTON (1919) Table 9	4	1,055	257	264	24.3	−0.7	0.74	50
KEMPTON (1919) Table 11	8	3,625	881	906	24.3	−0.7	1.42	62
Total, COLLINS and KEMPTON, and KEMPTON	198	102,429	24,500	25,607	23.9	−1.1	11.85	64
BRINK* (1925) Table 7	15	1,263	258	316	20.4	−4.6	5.59	80
BRINK, Table 8	13	3,569	855	892	24.0	−1.0	2.13	69
BRINK, Table 9	6	1,675	384	419	22.9	−2.1	2.93	100
BRINK, Table 10	7	1,717	399	429	23.2	−1.8	2.48	86
BRINK, Table 11	4	869	209	217	24.1	−0.9	0.92	50
BRINK, Table 12	6	939	195	235	20.8	−4.2	4.47	67
Total, BRINK	51	10,032	2,300	2,508	22.9	−2.1	7.11	78
KIESSELBACH and PETERSEN(1926) Table 5	39	21,284	5,101	5,321	24.0	−1.0	5.14	67
Same, Table 6	68	19,126	4,623	4,782	24.2	−0.8	3.91	59
Total, KIESSELBACH and PETERSEN	107	40,310	9,723	10,078	24.1	−0.9	6.06	63
Total all investigators	356	152,871	36,523	38,218	23.9	−1.1	14.85	65

*In table 7 the silks were allowed to grow extra long before pollination, while in table 8 normal silks were used for comparison. In table 9, 10, and 11, the pollen had been treated with ultraviolet light, and in table 12, the pollen had been desiccated before use.

DEVIATION FROM EXPECTED RATIOS

Several explanations have been offered to account for the deviations from expected ratios noted throughout the foregoing investigations. The totals for most groups of ears fertilized by segregating pollen have shown such consistent deficiency of waxy kernels as to appear significant. The suggestions by KEMPTON (1919) to account for the deficiency may be summarized as follows:

(1) The two types of gametes may form in unequal numbers.

(2) There may be a differential death rate of the gametes during their formation so that equal numbers do not function.

(3) Lack of vigor of the pollen carrying the factor for waxy causing slower growth of their pollen tubes.

(4) Differential selection of sperms by female gametes.

(5) Greater death rate of zygotes homozygous for waxy.

(6) Mistakes of kernel classification.

After the discovery of the difference in the chemical nature of the carbohydrate reserves of the pollen, BRINK and MACGILLIVRAY (1924) suggested that the deficiency of waxy kernels may be associated with a difference in the metabolism of the pollen leading to a slower growth of the pollen tubes carrying the waxy factor.

BRINK (1925) has indicated that there may be other factors operating in conjunction with waxy which causes a deviation in the number of waxy kernels by influencing the rate of pollen tube growth. Such a factor resulting in a deviation in the number of sweet kernels has been reported by JONES and MANGELSDORF (1925). WENTZ (1925) has also presented a case which may be accounted for by such factors.

In addition to the above possible explanations the following are offered:

(1) Differential retention of viability by pollen under the artificial conditions frequently accompanying control pollination. BRINK'S experiment in which the deficiency of waxy kernels was increased by desiccating the pollen over calcium chloride suggest differential viability in stored pollen.

(2) Accidental contamination by stray pollen.

(3) Since the starchy outweighed the waxy in paired segregating kernels on a number of ears examined, it would seem that the starchy pollen may also have a greater quantity of carbohydrate reserve, resulting in differential pollen tube growth.

CONCLUSIONS

An examination of all available data leads to the following conclusions:

The endosperm of certain maize has come to be known as *waxy* because of its peculiar appearance which distinguishes it from the endosperms of other types of maize. This is due to the presence of a carbohydrate which stains red with iodine, while ordinary starch stains blue.

This term, *waxy*, has been extended for convenience to this carbohydrate wherever found in other parts of the plants, and to the factor which determines it.

Waxy carbohydrate occurs in the pollen and embryo sacs as well as endosperms of waxy maize and so far as known is limited to these regions. The presence of this carbohydrate in these three regions appears to be determined by the same factor.

In crosses with starchy maize this waxy character behaves as a simple Mendelian recessive. The hybrid kernels have starchy endosperms. When planted no waxy carbohydrate appears in the plant until after the reduction division when equal numbers of gametophytes with the starchy or waxy carbohydrate (pollen and embryo sacs) are found as shown by the iodine test.

Kernel segregation for waxy endosperm occurs on ears produced by (1) homozygous waxy plants fertilized by segregating pollen, (2) plants heterozygous for waxy, fertilized by pure waxy pollen, and (3) plants heterozygous for waxy fertilized by segregating pollen.

In the first two combinations 50 percent waxy kernels would be expected and 25 percent waxy in the last case, if free from differential fertilization. However, the combined results of all investigators for those combinations in which segregating pollen was used show a slight deficiency of waxy kernels.

Of the various explanations that have been offered for this deficiency the most plausible would seem to be, (1) unequal fertilization resulting from differential pollen tube growth dependent upon the waxy factor or a linked factor, and (2) systematic errors connected with either the pollinations or with the kernel classification.

The theory of such differential pollen tube growth is supported primarily by the fact that when all results are combined, there is a deficiency of 0.7 percent waxy kernels from the expected 50 percent ratio and 1.1 percent deficiency from the expected 25 percent ratio when segregating pollen was used, whereas no deviation from expected ratios resulted when

pure waxy pollen and segregating ovules were used. The greater deficiency secured by BRINK with extra long silks tends further to substantiate this.

Based on probabilities the deviation from the expected ratio, if due to slower pollen tube growth associated with the waxy factor, should be twice as large when only the male parent is segregating as when both parents segregate. This is contrary to the results stated above and suggests that other causes are involved. This is further indicated by the fact that only 60 percent of the total combined number of 559 ears fertilized by segregating pollen showed a deficiency of waxy kernels, and also by the fact that certain rather large groups of such ears were free from deficiency.

The evidence at hand seems insufficient to definitely establish the causes or the significance of the deviations herein reviewed.

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THE EXPRESSION OF MENDELIAN FACTORS IN THE GAMETOPHYTE OF MAIZE

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DISTORTED RATIOS IN DEFECTIVE SEEDS

In 1918 a number of plants of a large-seeded, yellow, variety of flint corn, known as Gold Nugget, were self-pollinated. Nineteen selfed ears were secured and among these were three which segregated for a type of endosperm abnormality to which has been applied (JONES 1920) the term "defective seeds". Defective seeds may be described as lethal or semi-lethal characters in which the endosperm and embryo are greatly reduced in size or almost completely lacking. These characters appear very frequently when maize is inbred and it has been shown (MANGELSDORF 1923, 1926) that there are many genetically distinct types of this abnormality.

The defective which appeared in the Gold Nugget variety and to which the factor symbol *de*$_1$ has been given, is a semi-lethal type. The recessive seeds are characterized by a smaller size and aborted, shrivelled appearance. Their development is about half that of normal seeds on the same ears and they can, in most cases, be readily and accurately separated from the latter.

[1] Also contribution from the BUSSEY INSTITUTION of HARVARD UNIVERSITY.

In 1920 the strain carrying the de_1 factor was crossed with a defective from another variety. In the F_2 endosperm generation of this cross, three ears segregating for defective seeds of the de_1 type were obtained. These ears bore 567 normal and 194 defective seeds, almost a perfect 3:1 ratio. The natural conclusion was that this character is inherited as a simple Mendelian recessive.

In 1922 a de_1 strain of slightly different pedigree from the one used in 1920, but closely related to the latter, served as a pollen parent in crosses with five different unrelated stocks. In the F_2 generation of these crosses fourteen progenies segregating for the parental de_1 type were recovered.

TABLE 1

Progenies segregating for de_1 in the F_2 endosperm generation of crosses made in 1922.

EAR NO.	NORMAL SEEDS	DEFECTIVE SEEDS	DEVIATION	DEVIATION / P.E.	PERCENT DEFECTIVE
22	160	49	−3	.7	23.4
24	154	63	9	2.1	29.0
25	183	90	22	4.6	33.0
42	184	55	−5	1.2	23.0
126	428	126	−15	2.2	22.7
128	265	90	1	.2	25.4
129	369	180	43	6.3	32.8
173	200	93	20	4.0	31.7
388	498	197	23	3.0	28.3
389	424	155	6	.9	26.8
391	127	61	14	3.5	32.4
392	328	82	−21	3.6	20.0
393	284	97	2	.3	25.5
1520	346	175	45	6.8	33.6
Total	3950	1513	147	6.8	27.7

When the counts of normal and defective seeds on these ears were combined (table 1) it was found that the defectives occurred in excess of the theoretical expectation. The average percentage of defectives in this group was 27.7. This represents a deviation from a 3:1 ratio of 6.8 times the probable error and is undoubtedly significant. A deviation of this size should occur, by chance alone, only once in several hundred thousand trials. The excess of recessives is especially noteworthy because defectives of other types frequently show small deficiencies (MANGELSDORF 1926).

The reason for the difference between the percentage of recessives in the 1920 and 1922 crosses apparently lies in the different strains of de_1 which

were used as parents. Table 2 shows three successive generations of segregating ears of the original Gold Nugget strain. The proportion of normal to defective seeds in this strain had always approximated a 3:1 ratio and the average percentage of recessives for the entire group is 25.5. It is noted, however, that the two of the ten ears showed an excess of defectives of three or more times the probable error. It so happens that one of these excess ears, 105-9-8-2, was used as the pollen parent in the crosses made in 1922, before the significant deviation from a normal ratio had been noted. The 1920 cross, however, which gave normal segregation in F_2 was made with plants grown from ear 105-9-7 which itself segregated normally. Apparently the excess of recessives in the second generation of the 1922 crosses traces back to some condition which caused an excess in the parental ear.

TABLE 2

Segregating progenies of three successive generations of inbreeding of a "defective" strain of Gold Nugget.

EAR NO.	NORMAL SEEDS	DEFECTIVE SEEDS	DEVIATION	DEVIATION / P.E.	PERCENT DEFECTIVE
105-9	250	73	−8	1.5	22.6
105-9-3	117	49	8	2.2	29.5
105-9-4	159	46	−5	1.2	22.4
105-9-5	146	44	−3	.7	23.2
105-9-6	185	49	−9	2.0	20.9
105-9-7	243	75	−5	1.0	23.6
105-9-8	180	78	14	3.0	30.2
105-9-9	213	66	−4	.8	23.7
105-9-8-1	202	61	−5	1.1	23.2
105-9-8-2	134	84	30	7.0	38.5
Total and Average	1829	625	11	.8	25.5

The average percentage of defective seeds, 27.7 percent, in the fourteen ears shown in table 1 represents a significant excess but is not as great a deviation as occurred in the parental ear. When the ears in this group are considered separately, however, it is noted that seven of them show no significant departures from a 3 : 1 ratio; one shows a deficiency and the remaining six an excess greater than three times the probable error. Deviations of three times the error are expected, by chance alone, fairly frequently but in this case half of the ears show marked departures. Clearly the group is not a homogeneous one.

When the six ears which show significant plus deviations are combined, the average for the group is 31.9 percent recessives as compared to 25.1 percent for the non-deviating ears and 20.0 percent for the single ear with a significant minus deviation. In other words this lot of fourteen ears can be divided, on the basis of the deviations, into three distinct groups with *high*, *normal* and *low* defective ratios. A deviation of three times the probable error has been arbitrarily chosen as the dividing point.

If the *high* and *low* defective ears are merely the result of chance deviations, then progenies grown from such ears should give approximately normal ratios in F_3. If, however, these deviations have a genetic basis, distorted ratios might again be expected in F_3.

TABLE 3

Segregating F_2 progenies from "high defective" F_2 ear No. 391.

EAR NO.	NORMAL	DEFECTIVE	DEVIATION	DEVIATION / P.E.	PERCENT DEFECTIVE
Progenies with significant excess					
813	174	85	20	4.3	32.8
822	227	99	18	3.4	30.4
Total	401	184	38	5.4	31.5
Progenies with significant deficiency					
810	108	15	−16	4.9	12.2
811	319	63	−33	5.8	16.5
815	235	56	−17	3.4	19.2
Total	662	134	−65	7.9	16.8
Progenies with no significant deviation					
817	275	83	−7	1.3	23.2
819	130	55	9	2.3	29.7
820	273	89	−2	.4	24.6
Total	678	227	1	.1	25.1
Grand Total	1741	545	−27	1.9	23.8

Third generation progenies of two of the *high* defective ears, 391 and 1520, have been grown and the results are presented in tables 3 and 4.

Twelve progenies were obtained from ear 391 (32.4 percent recessives in F_2), of which eight were segregating for defective seeds. Although the average percentage of recessives for this group is approximately normal, 23.8 percent, it is noted that five of the eight ears deviate from a normal ratio by amounts greater than three times the probable error. Two ears are *high* with an average of 31.5 percent defectives, three are *low* with an

average of 16.8 percent recessives and the remaining three are *normal* with an average of 25.1 percent.

In connection with a possible explanation of the distorted ratios in waxy endosperm, presented later, particular attention is called to the difference between the percentage of defectives in the parental F_2 ear (32.4), and the average percentage of defectives in its progenies (23.8). The average of the F_3 progenies approached closely the normal expectation and it is only when the ears are considered separately that irregularities are noted.

Seventeen F_3 progenies were obtained from ear 1520 (33.6 percent recessives in F_2), of which ten were segregating. Four of the segregating ears were *high* with an average of 32.8 percent defectives, one *low*, with 16.8 percent, and five were *normal* with 25.3 percent defectives.

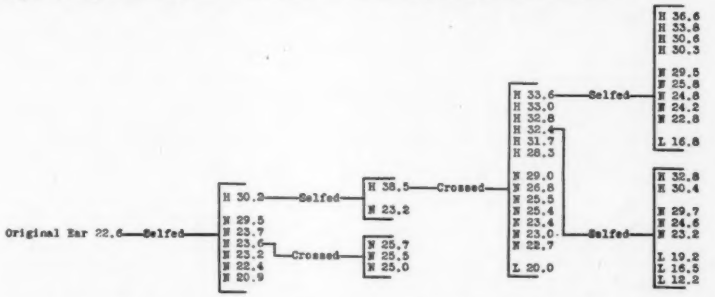

FIGURE 1. Pedigree record of the de_1 stock showing the inheritance of deviations from normal ratios. The letters H and L represent ears with plus or minus deviations greater than three times the probable error. N represents ears in which the deviation is less than three times the error.

Combining the 18 segregating progenies from these two high defective ears we find that six are *high* with an average of 32.4 percent recessives, four are *low* with 16.8 percent recessives and eight are *normal* with 25.2 percent recessives. The average for the entire F_3 group is 26.4 percent as compared to 33.3 percent for the two parental F_2 ears, a difference of practically seven percent between F_2 and F_3.

Obviously the marked departures from normal ratios in ten of the eighteen F_3 ears cannot be attributed to chance alone. Nor can the results be explained by faulty classification since errors of this sort are usually consistently in one direction. That the marked deviations have a genetic basis is indicated by the fact that they appear in four separate generations as is shown in figure 1.

Apparently the original stock contained a factor or factors which in some way disturbed the normal segregation. The action of this factor first

became apparent in the second generation of inbreeding. It has since been transmitted to later generations both in selfed lines of the original stock and in crosses.

TABLE 4

Segregating F_3 progenies from "high defective" F_2 ear No. 1520.

EAR NO.	NORMAL	DEFECTIVE	DEVIATION	DEVIATION / P.E.	PERCENT DEFECTIVE
Progenies with significant excess					
1788	224	99	18	3.5	30.6
1790	156	90	28	6.1	36.6
1792	365	186	48	7.1	33.8
1806	205	89	15	3.0	30.3
Total	950	464	109	10.0	32.8
Progenies with significant deficiency					
1794	188	38	−18	4.1	16.8
Progenies with no significant deviation					
1795	219	70	−2	.4	24.2
1796	167	58	2	.5	25.8
1797	85	28	0	..	24.8
1802	170	71	11	2.4	29.5
1803	227	67	−7	1.4	22.8
Total	868	294	4	.4	25.3
Grand Total	2006	796	96	6.2	28.4

INTERPRETATION OF DEFECTIVE RATIOS

Whenever marked deviations from expected ratios are encountered the possibility of lethal factors at once suggests itself. In maize, however, where a large number of seeds are arranged in a very regular fashion on a single inflorescence, lethal factors, or any sort of differential mortality of the zygotes are readily noted by the occurrence of aborted kernels scattered over the ear. The defective seed in question is, in fact, itself a semi-lethal character.

Lethal factors, moreover, should cause an excess of recessives only when they are linked with the dominant allelomorph of the recessive in question. The marked plus deviations in six of the F_2 ears shown in table 1 could be explained on the basis of lethal factors, only by assuming that each of the five separate seed parents involved in these crosses was heterozygous for a lethal factor linked with the dominant allelomorph of the de_1 gene. The possibility of lethal factors can, therefore, be safely ruled out.

The next hypothesis to be considered is that of differential pollen tube growth. The fact that defectives of other types had frequently given slight deficiencies suggested that these characters, which have such a profound deleterious influence on the sporophyte, might also have some effect in the gametophyte generation, particularly on the rate of pollen tube growth. To test this hypothesis a large number of ears segregating for various types of defectives were arbitrarily divided into upper and lower halves and the proportion of defectives in each half determined separately. If the two types of pollen tubes differ in their rate of growth it might be expected that the greater distance which the tubes were required to travel in reaching the ovules at the base of the ear, would react in favor of the faster growing tubes; that the longer the race the more certain the success of the faster individuals.

TABLE 5

Segregation in upper and lower halves of nine "high defective" ears.

EAR NO.	UPPER		LOWER		PERCENTAGE		GAIN IN LOWER HALF
	De	*de*	*De*	*de*	Upper	Lower	
25	75	35	108	55	31.8	33.7	1.9
129	179	83	190	97	31.7	33.8	2.1
173	101	49	99	44	32.7	30.8	−1.9
388	246	91	252	106	27.0	29.6	2.6
391	77	32	50	29	29.4	36.7	7.3
1788	108	45	116	54	29.4	31.8	2.4
1790	77	43	79	47	35.8	37.3	1.5
1792	189	92	176	94	32.7	34.8	2.1
1806	106	40	99	49	27.4	33.1	5.7
Total	1158	510	1169	575	30.6	33.0	2.4

The results of these counts indicated that, with most of the defective types, there was no appreciable difference between the upper and lower halves of the ear in proportion of defectives, and that the defective seed factors do not *per se* reduce the rate of pollen tube growth.

It happened, however, that several of the *high* and *normal* ears of the de_1 stock were included in this particular study, without realizing at the time that some of these ears were exceptional. The counts on these ears and several additional ones which have since been examined are shown in tables 5 and 6.

In the *high* ears the average percentage of defectives in the upper halves was 30.6 as compared to 33.0 in the lower halves of the same ears. This difference, though not great, appears to be significant, the odds against its chance occurrence being about 70 to 1 (calculated by Student's formula). The fact that the difference is consistent, all the ears in table 5 with one exception showing a greater proportion of recessives in the lower halves, is regarded as particularly important.

The *normal* ears on the other hand (table 6) show differences in favor of the upper halves as frequently as the lower and the averages of the two halves are very closely alike, 24.6 and 25.5 percent. These results, so far as they go, point to differential rate of pollen tube growth in the *high* ears and equal rate of pollen tube growth in the *normal* ears.

TABLE 6

Segregation in upper and lower halves of seven "normal defective" ears.

EAR NO.	UPPER		LOWER		PERCENTAGE		GAIN IN LOWER HALF
	De	*de*	*De*	*de*	UPPER	LOWER	
22	84	27	76	22	24.3	22.4	−1.9
24	75	32	79	31	29.9	28.2	−1.7
42	91	27	93	28	22.9	23.1	.2
126	211	54	217	72	20.4	24.9	4.5
128	142	52	123	38	26.8	23.6	−3.2
389	235	75	189	80	24.2	29.7	5.5
393	160	59	124	38	26.9	23.5	−3.4
Total	998	326	901	309	24.6	25.5	.9

Apparently the differential rate of growth is not due to the action of the de_1 factor itself. If pollen tubes carrying this gene were regularly faster or slower in rate of growth, the deviations should regularly occur in the same direction in *all* of the ears. This is not the case. Some ears have marked minus deviations, some marked plus deviations and the remainder segregate normally.

These peculiar results may, however, be explained by assuming that the chromosome pair which carries the De_1 and de_1 factors also bears a gene which influences the rate of pollen tube growth. Since this hypothetical factor has its expression in the gametophyte generation we may give it the factor symbol *Ga*. This factor speeds up the rate of growth in the pollen tubes which carry it and, in the original stock, was linked with de_1, thus enabling a larger proportion of de_1 gametes to accomplish fertilization than would occur by chance alone.

On this assumption the *high* defective plant which served as pollen parent for the 1922 crosses was of the genetic composition $Gade_1$, $gaDe_1$. Such a plant would produce four kinds of gametes, *Gade*, *gaDe*, *gade*, and *GaDe*. The seed parents of these crosses contributed only one kind of gamete *gaDe*. Thus four distinct classes of F_1 plants would be produced as follows:

1. *gaDe GaDe*
2. *gaDe gaDe*
3. *gaDe Gade*
4. *gaDe gade*

These four types would not be expected to occur in equal numbers because their proportion depends upon the amount of crossing over between *Ga* and *de* in the original stock.

Classes 1 and 2 are homozygous for the dominant De_1 factor and need not be considered further since they do not segregate for defective seeds. Plants in class 4 give segregating ears but the ratios should be normal because the plants are homozygous for *ga* and no differential pollen tube growth can occur. Plants of class 3, however, should, when selfed, give only *high* defective ears because both factors are in the heterozygous condition and the *Ga* factor which speeds up the rate of pollen tube growth is linked with the de_1 factor which causes defective seeds, thus enabling a larger proportion of the de_1 gametes to accomplish fertilization than would occur by chance alone.

A glance at table 1 shows that this is exactly the situation in the fourteen F_2 ears. Seven *normal* and six *high* ears resulted from these crosses. In addition, however, there is one *low* ear. *Low* ears are not expected from these particular crosses and it is not certain whether ear 392 with 20.0 percent recessives is an exception to theory or merely a chance variation from a *normal* ratio.

It is obvious that in dividing the ears into *high*, *low* and *normal* on the basis of the size of their deviations, some ears which have plus or minus deviations due to chance alone and not to genetic factors, will be included in the two extreme groups. Also some ears which are genetically *high* or *low* will be classed as *normal* because the number of seeds counted is not sufficient to establish the significance of the deviations.

What should happen in F_3 when the F_2 seeds from a *high* defective ear are grown? The *high* ear, we assume, was borne on a plant of the composition *Gade gaDe*. Such a plant would form four kinds of gametes in the pollen as well as in the ovules and self-pollination should give ten classes of

seeds. The genetic constitution of these ten classes and their theoretical behavior when grown and self-pollinated is shown below:

GENOTYPE	BEHAVIOR
1. *GaDe GaDe*	Does not segregate
2. *GaDe gaDe*	Does not segregate
3. *gaDe gaDe*	Does not segregate
4. *GaDe Gade*	Segregates; 3 : 1 ratio
5. *gaDe gade*	Segregates; 3 : 1 ratio
6. *Gade gaDe*	Segregates; *high* defective ratio
7. *GaDe gade*	Segregates; *low* defective ratio.
8. *Gade Gade*	Defective seed
9. *Gade gade*	Defective seed
10. *gade gade*	Defective seed

Genotypes 1, 2, and 3 are homozygous for De_1 and do not segregate. Genotypes 8, 9, 10 are defective seeds and will not grow. Genotypes 4 and 5 segregate but both give normal ratios because the plants are homozygous for *Ga* or *ga* and differential pollen tube growth cannot occur. Plants in genotype 6 are heterozygous for both factors and, since *Ga* and de_1 are linked, should give *high* defective ears. Genotype 7 represents a new combination in which the linkage relations have been reversed. Here the *Ga* factor is linked with the dominant allelomorph and instead of an excess of defectives we should expect a deficiency.

Briefly summarized, then, the seeds from a *high* F_2 ear should, when grown and selfed, give *high*, *low*, and *normal* F_3 progenies. The 18 segregating ears in tables 3 and 4 meet these requirements very satisfactorily. Six are *high*, eight are *normal* and four are *low*. The ratio in which these three types of segregating progenies appear depends not only on the amount of crossing over between *Ga* and de_1 but on the ratio in which the *Ga* and *ga* gametes are presented at fertilization.

It should be emphasized that the validity of this interpretation does not necessarily depend upon the assumption of differential pollen tube growth. Any factor which affects the proportion in which the gametes are presented at fertilization would act in the same manner although its effect might be produced in a number of ways, as for example, by causing differences in the duration of viability or resistance to unfavorable moisture and temperature extremes.

The important fact is that this stock apparently *does* carry a factor which disturbs normal segregation and that this factor is transmitted from generation to generation as is any other Mendelian character. *How* the factor produces its disturbances is another question and one which seems to be partially answered by the data which point to differential pollen tube growth.

DISTORTED RATIOS IN SUGARY ENDOSPERM

The hypothesis of an accessory factor causing differential rate of pollen tube growth has been adopted because the situation is almost identical to that encountered when sugary endosperm is crossed with certain small seeded corneous varieties of the *Zea maize everta* type, known as Squirrel Tooth or Rice Pop.

Although sugary is usually inherited as a simple recessive and gives ratios closely approximating expectation, the Rice Pop × sugary crosses are exceptional in that they always produce a marked deficiency of sugary seeds in the F_2 endosperm generation. This deficiency was first noted by CORRENS (1902) who reported 16 percent sugary seeds in the second generation. EAST and HAYES (1911) also noted the same peculiarity and suggested that the disturbance might be due to faulty classification or to unknown characters contributed by the parents. JONES (1924) and EMERSON (1925) have both made detailed studies of this cross and both have come to the conclusion that the aberrant ratios may be attributed to differential pollen tube growth. EMERSON, however, suggested that the differential rate of growth is not due to the *Su* gene itself but to an accessory factor on the same chromosome. The details of EMERSON'S interpretation of this cross have not yet been published and it is not known whether his explanation agrees with the one given here.

The results of the genetic studies of the sugary × pop cross at the CONNECTICUT STATION are briefly summarized in the following paragraphs.

An inbred strain of pointed pop corn was crossed with a first generation hybrid of two inbred strains of sweet corn. One of the sweet strains was a small yellow-seeded variety known as Golden Bantam, the other a large white-seeded variety known as Evergreen. This material was used because it had shown a high degree of selective action in pollen mixtures where pollen from two different types of plants, acting in competition, fertilized more of the ovules of its own type than of the diverse types as previously reported by JONES. (1920, 1922).

In a total of 3681 seeds from eight self-pollinated F_1 plants of this cross shown in table 7, there are only 16.2 percent of sugary seeds. This represents a departure from the normal ratio of 18.3 times the probable error. The percentages of recessives in the individual ears range from 10.5 to 21.4 and the deficiency is greater than three times the probable error in all but one of the ears. In this ear the deviation is 2.6 times the probable error.

F_1 plants of similar cross, though of somewhat different ancestry were backcrossed both ways with the recessive parent. In the backcross of the heterozygote with pollen from the recessive parent (*Susu* × *susu*), 1374

starchy and 1397 sugary seeds were produced. In the reciprocal backcross (*susu* × *Susu*) 885 starchy and 939 sugary seeds occurred. The deviation from a 1 : 1 ratio is not significant in either case. In this respect the results differ from observations with waxy corn which are discussed later. In all this material the segregation of smooth, corneous seeds and wrinkled, glassy seeds was very clear-cut. Occasionally a recessive seed was found that was not deeply wrinkled and at first glance might be classed as a dominant. However, the opportunity for faulty classification was just as great in the F_1 plants backcrossed with the recessive as in the F_1 plants self-pollinated. In the one case a marked deviation was found; in the other, not.

TABLE 7

F_2 progenies of a cross between sugary endosperm and Squirrel Tooth Pop.

EAR NO.	STARCHY	SUGARY	DEVIATION	DEVIATION / P.E.	PERCENT SUGARY
(*Su*×*su*) −1	366	43	−59	10.0	10.5
2	346	94	−16	2.6	21.4
3	376	65	−45	7.3	14.7
(*su*×*Su*) −1	322	56	−39	6.9	14.8
2	378	77	−37	5.9	16.9
3	421	98	−32	4.8	18.9
4	391	47	−63	10.3	10.7
5	485	116	−34	4.8	19.3
Total	3085	596	−324	18.3	16.2

The next step was to backcross the F_1 plants with the dominant parent (*Susu* × *SuSu* and *SuSu* × *Susu*). Since the result of fertilization by a recessive or dominant carrying gamete would be obscured, because all the seeds would have the dominant endosperm condition, it was necessary to grow the plants and determine which were segregating and which were not. This was done by planting the backcrosses, made both ways, and allowing all of the plants to interpollinate naturally. Any plants that resulted from *Su* × *Su* fertilization would show no recessives, while most of the plants of the composition *Su* × *su* would be expected to show some recessive seeds. With no selective action one-half of the plants would be segregating so that one-fourth of the pollen in the field would be carrying the recessive factor. Segregating plants would be expected to have half of their ovules with the recessive factor so that segregating ears should have, on the average, one-eighth of their seeds recessive. Selective pollination working against the recessive gametes would reduce this proportion. Only one ear was taken from each plant and most of these contained several hundred

kernels. All ears which had any sugary seeds were classed as segregating. The number of sugary seeds varied on different ears but the number of plants which had no recessive seeds and for that reason would be wrongly classified was probably less than seven percent and was not likely to be enough to disturb the results seriously in view of the wide departure from the normal 1 : 1 ratio obtained. The backcross of pollen from the homozygous dominant parent on the heterozygous F_1 plants (*Susu* × *SuSu*) gave 207 segregating and 213 non-segregating individuals. These numbers differ from a 1 : 1 ratio less than the probable error. But the pollen from the heterozygous F_1 plants backcrossed on the homozygous dominant parent (*SuSu* × *Susu*) gave 88 segregating and 353 non-segregating individuals, a deviation from a 1 : 1 ratio of 18.7 times the probable error.

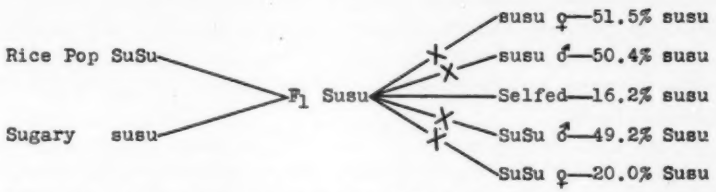

FIGURE 2. Diagram showing the behavior of the Rice Pop×Sugary cross when selfed and backcrossed reciprocally with both parents.

To sum up, the F_1 plants self-fertilized showed a selective action such that more of the gametes carrying the dominant factor united than would be the case in random mating. The heterozygous F_1 plants backcrossed with the recessive parent showed no selective action either way the pollinations were made. Backcrossed with the dominant parent there was no selective action when the pollen was alike but the pollen from the heterozygous F_1 plants showed a markedly greater pollinating ability on the part of the gametes carrying the dominant factor. These results are shown graphically in figure 2.

This series of facts shows that the selective action is not due to differences in the functioning of the pollen alone, although the differential action is exhibited only when the pollen is diverse, otherwise unequal numbers would be obtained in the backcross on the recessive parent as well as on the dominant.

As a result of these experiments JONES (1924) concluded that: "There is apparently an interaction between the pollen tube and the tissues in which it grows, such that pollen carrying the dominant factor is better able to accomplish fertilization than the pollen carrying its recessive allelomorph,

only in a sporophyte which also has the dominant factor either in the haploid or diploid state."

At that time it was thought that the *Su* factor itself was responsible for the speeding up of the pollen tubes in certain combinations. It now seems more probable that the results are due to linkage of the *Su* factor with a gametophyte factor (*Ga*). If the linkage relations between *Su* and *Ga* were reversed the results would be entirely different but the above statement as applied to the *Ga* factor would still hold.

The factor symbol for the gametophyte factor has been written in capital letters as though it were a true dominant. It may be questioned whether this is legitimate since the *Ga* factor has its expression in the gametophyte where it necessarily occurs in a haploid condition and is not required to compete with its allelomorph. The *Ga* pollen tubes are given an advantage, however, only when the sporophytic tissue in which they grow also has this factor, and the fact that in the sporophyte it is apparently as effective when heterozygous as when homozygous indicates that it is a dominant factor.

On the basis of a gametophyte factor linked with the *Su* factor, the combinations here discussed may be given the following factorial representation:

TYPE OR COMBINATION	COMPOSITION OF PLANT	MALE GAMETES
Rice Pop parent	*SuSuGaGa*	*SuGa*
Sugary parent	*susugaga*	*suga*
F_1 selfed	*SusuGaga*	*SuGa Suga suGa suga*
$F_1 \times$ sugary parent	*SusuGaga*	*suga*
Sugary parent $\times F_1$	*susugaga*	*SuGa Suga suGa suga*
$F_1 \times$ starchy parent	*SusuGaga*	*SuGa*
Starchy parent $\times F_1$	*SuSuGaGa*	*SuGa Suga suGa suga*

With this factorial composition in mind the results reported in the preceding paragraphs are readily understood on the assumption that the *Ga* factor is effective in speeding up pollen tube growth only in a sporophyte which has the *Ga* factor in the homozygous or heterozygous state.

The F_1 selfed shows a selective action because *Ga* and *ga* pollen tubes compete with each other in sporophytic tissue which has the *Ga* factor in the heterozygous condition. The *Ga* tubes are given an advantage under these conditions and since *Ga* and *Su* are linked, more *Su* than *su* gametes, reach the micropyle and accomplish fertilization. F_1 plants pollinated by the sugary parent show no selective action because only one type of male gamete is involved. The backcross of the sugary parent by the F_1 involves four types of male gametes but no selective action is shown because the sporophyte in which the pollen tubes must grow lacks the dominant *Ga*

factor. F_1 backcrossed by the starchy parent shows no selective action because only one kind of male gamete is present. But the backcross of the starchy parent by the F_1 again shows a very marked selective effect because *Ga* and *ga* gametes are competing in sporophytic tissue which has the dominant *Ga* factor in the homozygous state.

The F_2 progenies which show such a marked deficiency of sugary seeds, should, when selfed, produce the same three types of segregating progenies as did the *high* defective ears when selfed. Here of course the proportion of the three types would be reversed and (1) the majority should be deficient in sugary seeds, (2) some should segregate normally, and (3) a few should give an excess. EMERSON has grown such progenies and has obtained exactly these results, his *low* ears giving 15 percent recessives, his *high* ears 35 percent. The writers have also grown a few F_3 progenies from this cross and have obtained the expected types, although in the case of the *high* sugary ears the number of seeds counted was not great enough to prove that the deviations were statistically significant.

LINKAGE BETWEEN *Su* AND *de*$_1$

The question at once arises whether the deviations found in the *de*$_1$ stock are caused by the same accessory factor, always present in Rice Pop, which causes such marked disturbances in the starchy: sugary ratio. To determine this point crosses of *high* defective × pop, *low* defective × pop, *high* defective × sugary, and *low* defective × sugary have been made and the F_2 endosperm generation of these crosses will be available in another season. In the meantime, indirect evidence that the accessory factor in both stocks is probably the same is available in a cross of *normal* defective × sugary, which had been made for another purpose. Four F_2 progenies of this cross are shown in table 8.

It is noted that the two parental classes are in excess, while the two new combinations show a deficiency. When parental classes are combined and compared to the new combinations in the form of a 10 : 6 ratio, the deviation is found to be 4.9 times the probable error. Such a deviation should occur by chance alone only once in 1052 trials but can readily be explained by assuming linkage (repulsion phase) between *su* and *de*$_1$. The percentage of crossing over as determined from the normal seeds is 38.5; determined from the defective class it is 39 percent.

RELATIVE EFFECTIVENESS OF *Ga* AND *ga* GAMETES

Knowing that the *de*$_1$ and *su* factors are linked and that both are affected to a marked degree by an accessory factor located on the same chromo-

some, it is almost certain that the same accessory factor is acting in both stocks. On such an assumption it is possible to estimate approximately the position on the chromosome of the *Ga* factor in relation to the *su* and de_1 genes and at the same time to determine roughly the ratio of *Ga* to *ga* gametes which accomplish fertilization.

The presence of gametophyte factors which disturb the equality in which two kinds of gametes are presented at fertilization, brings up entirely new problems in Mendelian segregation. The amount of crossing over between a gametophyte factor and a sporophyte factor may range from 0 to 50 percent. At the same time the ratio in which the *Ga* and *ga* gametes occur at fertilization may range from 1 : 1 to 1 : 0.

TABLE 8

Segregation in F_2 of a cross between sugary endosperm and defective seed.

EAR NO.	NORMAL SEEDS		DEFECTIVE SEEDS	
	Starchy	Sugary	Starchy	Sugary
947	71	36	42	10
951	213	89	75	27
953	219	81	85	18
956	98	32	45	9
Total	601	238	247	64
Ex. 9 : 3 : 3 : 1	647	216	216	72
Deviation	−46	22	31	−8

Grouping into a 10 : 6 ratio

Found	665	485
Ex. 10 : 6	*719*	*431*
Deviation	54 ± 11.1	
Dev./P. E.	4.9	

The amount of crossing over and the relative effectiveness of the accessory factor are, then, the two variables which determine the size of the deviations from normal ratios. Table 9 shows the theoretical percentage of recessives which should occur with different combinations of these two variables, assuming that a gametophyte factor *G* which increases the chances of the gametes, in which it occurs, to accomplish fertilization, is linked with the dominant allelomorph *S* of a recessive sporophyte factor. If the linkage were reversed (*Gs gS*), the deviations would be of the same magnitude but would occur in the opposite direction. The size of the deviations produced by different combinations of the two variables is also illustrated in figure 3.

The average percentage of defective seeds in the *high* ears of tables 3 and 4 is 32.4, a deviation of 7.4 percent from normal. The *low* ears from the

same families average 16.8 percent recessives, a deviation from normal of 8.2 percent. The average deviation for both groups is 7.8 percent. In the *low* sugary ears of table 7 the average deviation from a normal ratio is 8.8 percent.

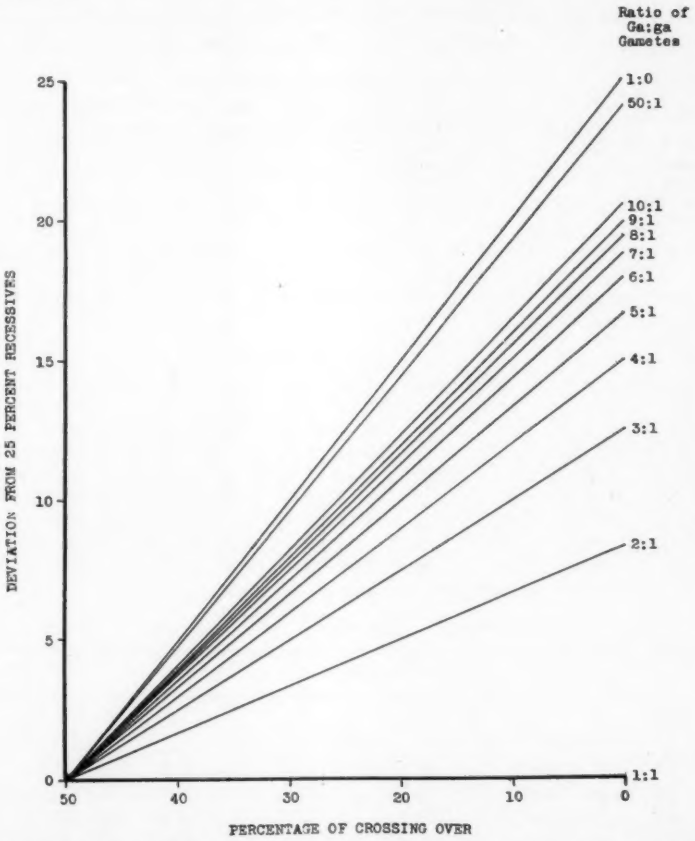

FIGURE 3. Diagrams showing the theoretical deviations from 25 percent recessives resulting from various combinations of crossing over and relative effectiveness of two kinds of gametes in accomplishing fertilization.

A deviation of 7.8 percent in the defective seed ratios and 8.8 percent in the sugary ratios might be caused by any number of combinations of crossing over and differential effectiveness of the *Ga* and *ga* gametes. This is illustrated by the curves in figure 3. Thus if the value of one of these

influences were known it would be a fairly simple matter to determine the value of the other. Unfortunately this is not the case.

It is known, however, that the crossing over between *Su* and de_1 is approximately 39 percent. An accessory factor could affect the two almost equally, only if it were approximately the same distance from both. This means that its locus must lie between the *Su* and de_1 loci.

TABLE 9

Percentages of recessives (ss) expected when a factor G which stimulates the rate of pollen tube growth is linked with the dominant allelomorph S of a recessive character s.

RATIO IN WHICH GAMETES ARE PRESENTED	PERCENTAGE OF CROSSING OVER BETWEEN *G* AND *S*									
	0	5	10	15	20	25	30	35	40	45
1 : 1	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
2 : 1	16.65	17.50	18.33	19.17	20.00	20.83	21.67	22.50	23.33	24.17
3 : 1	12.50	13.75	15.00	16.25	17.50	18.75	20.00	21.25	22.50	23.75
4 : 1	10.00	11.50	13.00	14.50	16.00	17.50	19.00	20.50	22.00	23.50
5 : 1	8.33	10.00	11.67	13.33	15.00	16.67	18.33	20.00	21.67	23.33
6 : 1	7.14	8.93	10.72	12.50	14.29	16.07	17.86	19.64	21.43	23.22
7 : 1	6.25	8.13	10.00	11.88	13.75	15.63	17.50	19.37	21.25	23.12
8 : 1	5.56	7.50	9.45	11.39	13.33	15.28	17.22	19.17	21.11	23.06
9 : 1	5.00	7.00	9.00	11.00	13.00	15.00	17.00	19.00	21.00	23.00
10 : 1	4.55	6.59	8.64	10.68	12.73	14.77	16.82	18.86	20.90	22.95
20 : 1	2.38	4.64	6.91	9.17	11.43	13.69	15.95	18.21	20.48	22.74
30 : 1	1.61	3.95	6.29	8.63	10.97	13.31	15.64	17.98	20.32	22.66
40 : 1	1.22	3.60	5.98	8.35	10.73	13.11	15.49	17.87	20.24	22.62
50 : 1	.98	3.38	5.78	8.19	10.59	12.99	15.39	17.79	20.20	22.60
1 : 0	.00	2.50	5.00	7.50	10.00	12.50	15.00	17.50	20.00	22.50

TABLE 10

Crossing over values and map distances indicated by ordinates erected at different points on abscissa in figure 4.

POINT ON ABSCISSA	PERCENTAGE CROSSING OVER		MAP DISTANCE		TOTAL MAP DISTANCE
	Ga — *Su*	*Ga* — de_1	*Ga* — *Su*	*Ga* — de_1	
3.7	19.3	22.7	20.9	25.0	45.9
3.8	19.8	23.1	21.5	25.5	47.0
3.9	20.3	23.5	22.1	26.0	48.1
4.0	20.8	23.9	22.7	26.5	49.2
4.1	21.2	24.3	23.2	27.0	50.2
4.2	21.5	24.7	23.6	27.5	51.1
4.3	21.8	25.0	23.9	27.9	51.8
4.4	22.1	25.3	24.2	28.3	52.5

The map distance between *Su* and de_1 is 50 units as determined from HALDANE'S table (HALDANE 1919). These tables are, of course, based on the results from the sex chromosome in Drosophila but since they have

been found to apply equally well to the other chromosomes of Drosophila and to the linkage groups in Primula, it is probable that they hold for the linkage groups in maize.

The problem in this case is to determine at what point on the abscissa in figure 4 an ordinate can be erected which crosses the two curves at points, whose values converted into map distances and combined, equal 50 units. Table 10 shows the results of erecting ordinates at a number of points along the abscissa.

It is noted that an ordinate erected at 4.1 crosses the two curves at points whose values best fit the facts. In other words, if 4.1 times as many

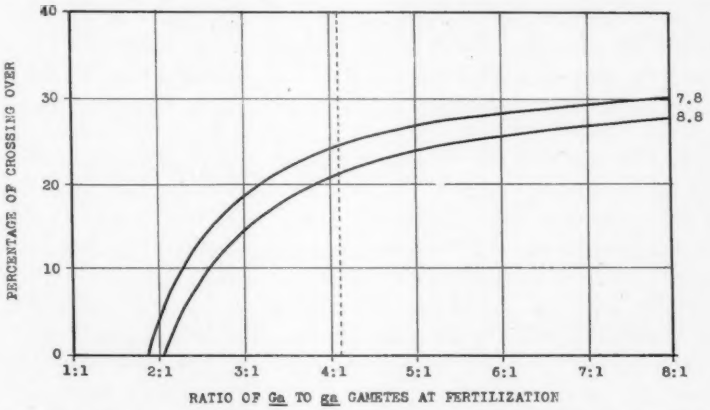

FIGURE 4. The various combinations of crossing over and relative effectiveness of two kinds of gametes which might cause deviations of 7.8 and 8.8 percent from the expected 25 percent recessives. An ordinate erected at 4.1 crosses the two curves at points whose values converted into map distances and added, equal the map distance between de_1 and *Su*.

Ga as *ga* gametes accomplish fertilization, then the crossing over between *Ga* and *Su* is 21.2 percent and between *Ga* and de_1, 24.3 percent. The map distance between *Ga* and *Su* would then be 23.2 units and between *Ga* and de_1, 27.0 units. These two values combined equal 50.2 units which is approximately the map distance between *Su* and de_1.

It is realized, of course, that it is impossible to arrive at more than a crude approximation by this method. It is difficult to determine the true value of either of these influences until one of them is more accurately known. Furthermore, the assumption has been made that the relative proportion of *Ga* and *ga* gametes which accomplish fertilization is always the same. This is probably not strictly true. The data already given, and

further data from mixed pollinations presented later, indicate that the selective action is due to differential pollen tube growth. The ratio of the two classes of gametes at fertilization would, therefore, depend to some extent on length of styles, weather conditions, and perhaps many other factors. In the long run these various influences would be expected to balance each other and over a period of years the ratio of the two types of gametes would be relatively constant. In comparing two series of observations, however, a variation in the relative effectiveness of the *Ga* and *ga* gametes must be kept in mind.

PREDICTING THE SIZE OF THE DEVIATIONS

If these approximations have any value it should now be possible to predict the deviations which will occur when other characters on the sugary chromosome are crossed with Rice Pop, providing that the amount of crossing over between sugary and the new character is known. A recent paper by WENTZ (1925) includes data which answer this purpose admirably. WENTZ found that a defective seed type de_{su} is linked with sugary with 3.2 percent crossing over. Since the crossing over between the *Ga* and *Su* loci is approximately 21.2 percent, then the crossing over between *Ga* and de_{su} should be about 23.8 or 18.6 percent depending on the direction of the de_{su} gene from the sugary locus. With the *Ga* and *ga* gametes occurring at fertilization in the ratio of 4 to 1, a cross between Rice Pop (*GaDe GaDe*) and WENTZ'S defective (*gaDe gade*) should produce either 17.1 or 15.6 percent recessives on the segregating ears. Fortunately, WENTZ has crossed his defective seed strain with a stock tracing back to Rice Pop ancestry and apparently heterozygous for the accessory factor of the Pop parent. Eight segregating ears in the F_2 endosperm generation of this cross gave an average of 19.8 percent defective seeds as is shown in table 1 of WENTZ'S paper. This value differs from the value predicted above by 2.7 percent.

Through the kindness of Professor WENTZ the detailed data on the individual ears of this cross, which were not included in his published paper, were made available to us. These data showed that three of the eight ears gave approximately normal ratios averaging 24.7 percent recessives. The other five ears, however, all deviated from a normal ratio by significant amounts and when combined gave a total of 1475 normal and 309 defective seeds, an average of 17.3 percent recessives.

The agreement of this value with the 17.1 percent recessives predicted by the above calculations is surprisingly good and is either a remarkable coincidence or an excellent bit of evidence in substantiation of our hypothesis.

The position of the factors on the sugary chromosome is, then, approximately as shown in one or the other of the two following diagrams.

de_1		*Ga*	*Su*	de_{su}	*Tu*
de_{su}	*Su*	*Ga*	*Tu*	de_1	

Which of these arrangements is the correct one will be determined when the character *tunicate ear* is crossed with Rice Pop. The crossing over between *su* and *Tu* is approximately 28.6 percent (EYSTER 1921). In the first arrangement *Tu* is so far from *Ga* that its segregation would be affected only very slightly by the differential pollen tube growth. In the second arrangement *Tu* is fairly close to *Ga* and if this is the correct one, marked deviations should occur in the tunicate ratios when this character is crossed with Rice Pop or other stocks carrying the *Ga* factor.

GAMETOPHYTE FACTORS AND MIXED POLLINATIONS

The presence in Rice Pop of a gametophyte factor which influences the rate of pollen tube growth in sporophytic tissue of the same genetic constitution, probably accounts to a large extent for the results obtained in mixed pollination experiments. (JONES 1920, 1922). In these tests where a mixture of the white pop type and a yellow seeded sugary type was applied to the silks of both, there was a marked preference of the plants for their own kind of pollen. The results of five such mixed pollinations are shown in table 11.

TABLE 11

The amount of selective action shown by maize in five pollen mixtures.

POLLEN MIXTURE NO.	NUMBER OF SEEDS				TOTAL NO. OF SEEDS	DEVIATION FROM PERFECT PROPORTION IN PERCENT
	A×A	A×B	B×A	B×B		
1	811	11	381	2006	3209	41.35
2	4222	27	466	1404	6119	37.22
3	1568	2	319	224	2113	20.56
4	1930	29	73	309	2341	39.71
5	4084	6	963	290	5343	11.50

If there were no differences in pollinating ability, the proportion should be a perfect one within the limits of random sampling. The white smooth-seeded plants are designated as A in the tables and the plants with yellow, sugary seeds are listed under the heading of B. Without selective action the ratio of crossed to selfed seeds should be the same on ears of both types, regardless of the amount or viability of each kind of pollen. The last

column gives the deviations from the closest calculated perfect proportion based upon the percentage of cross-fertilized and self-fertilized seeds on each type of plant. The maximum deviation of 50 percent would indicate complete functioning of each kind of pollen on its own flowers to the exclusion of the foreign type. The deviations actually range from 14 to 41 in favor of self-fertilization. In some tests the deviations have been even higher but only the above five groups are given here because these were also used in determining differences in pollen tube growth.

That the selective action is due, in part at least, to differences in pollen tube growth, is indicated by the data in table 12. The ears were arbitrarily divided into top and bottom halves before shelling. The distance that the pollen tubes had to travel differed considerably in the case of seeds produced at the tip of the spike as compared with those at the base. If the plant's own pollen tubes grew more rapidly than the foreign tubes, we would expect fewer cross-fertilized seeds at the base than at the tip of the spike.

TABLE 12

The ratio of crossed seeds in upper and lower halves of ears resulting from mixed pollinations.

POLLEN MIXTURE NUMBER	PLANT TYPE	TOTAL NO. OF SEEDS	NO. OF CROSSED SEEDS PER 100		RATIO OF CROSSED SEEDS
			TOP	BOTTOM	TOP : BOTTOM
1	Pop	822	1.99	.54	3.7 : 1
1	Sugary	2387	17.23	14.64	1.1 : 1
2	Pop	4249	1.22	.05	24.4 : 1
2	Sugary	1870	26.66	23.01	1.2 : 1
3	Pop	1570	.25	.00	.25 : 0
3	Sugary	543	60.07	57.14	1.1 : 1
4	Pop	1959	2.54	.32	7.9 : 1
4	Sugary	382	24.73	13.78	1.8 : 1
5	Pop	4090	.10	.20	.5 : 1
5	Sugary	1253	78.43	75.35	1.1 : 1
Total and Average	Pop	12,690	1.22	.22	5.6 : 1
	Sugary	6,435	41.42	36.78	1.1 : 1

The last column in table 12 shows the ratio of crossed seeds in the top halves to those in the bottom halves. In all but one mixture there was a greater proportion of crossed seeds in the top halves of the ears and in this one exception there were only six crossed seeds in a total of four thousand. It is also noted that the excess of crossed seeds in the upper part of the inflorescence is much greater on ears of the pop type than those of the sugary type. In the former there were 5.6 times as many crossed seeds in the top halves while in the sugary ears there were only 1.1 times as many crossed

individuals in the upper part of the inflorescences. The marked selective action found in these mixtures is apparently due to a condition in the pop parent which permits the plant's own pollen tubes to grow more rapidly in its own styles. That this condition is brought about by a gametophyte factor on the sugary chromosome has already been indicated.

It will be noted that the data also indicate a slight preference of the sugary plants for their own kind of pollen. Probably there are many genetic factors which affect pollen tube growth and any of these which cause increased growth when the sporophytic tissue is of the same genetic constitution, will give the plants' own pollen an advantage over foreign pollen. Almost all pollen mixtures show a slight selective action in favor of the plants' own pollen but only where one of the types possesses a factor which causes a marked increase in the rate of pollen tube growth, such as is found in Rice Pop, is the selective action so decided.

DISTORTED RATIOS IN WAXY ENDOSPERM

The deficiency of waxy seeds originally noted by COLLINS and KEMPTON (1911) in the F_2 endosperm generation of hybrids between waxy and other varieties of maize can also be explained, at least partially, by the action of one or more gametophyte factors linked with the *Wx wx* pair.

New interest in the inheritance of waxy has recently been aroused by the discovery that the carbohydrate reserve in the waxy endosperm is of a different chemical nature than that of other varieties of maize, staining red with iodine, while other types of endosperm stain a deep blue (WEATHERWAX 1922), and that this difference in reaction to staining is also apparent in the pollen grains (BRINK and MACGILLIVRAY 1924, DEMEREC 1924, LONGLEY 1924) and in the embryo sac. (BRINK 1925, KIESSELBACH and PETERSEN 1926)

KIESSELBACH and PETERSEN (1926) have summarized all the available data on the inheritance of waxy endosperm, including considerable new data of their own. When all the results are combined there is a deficiency of 1.1 percent from the expected 25 percent when the heterozygote is selfed and .7 percent from the expected 50 percent when segregating pollen from heterozygous plants is applied to the pure recessives. These writers conclude that "the evidence at hand seems insufficient to definitely establish the causes or significance of the deviations", although the deviation in the first case is 14.8 times the probable error and in the second 5.4 times the error.

The difficulty of interpreting the waxy situation is not due to a deficiency of data but to the fact that data, which are not strictly comparable,

have been combined. Different investigators have used different stocks in crossing with waxy, and the waxy stock itself after repeated crossings and extractions, since it was first brought from China, is probably far from homogeneous. If accessory factors are involved in the inheritance of waxy it will be readily seen that the procedure of combining data from a wide variety of sources and especially that of combining different generations, is not a sound one. Merely adding a few thousand individuals to the third of a million already counted will contribute very little toward solving the cause of the deviations.

The fallacy of combining the data from separate generations is well illustrated by the defective seed ratios in table 3. An F_2 ear with 32.4 percent recessives produced F_3 progenies with an average of 23.8 percent recessives. At first glance it appeared that the deviation in F_2 was not inherited. Closer examination, however, shows that although the average percentage of defective seeds in F_3 is almost normal, the individual progenies show marked deviations, some in one direction, some in another.

ANALYSIS OF KEMPTON'S DATA

That a somewhat similar situation exists in the waxy stocks is shown by data from KEMPTON (1919). KEMPTON'S F_2 progenies gave an average of 23.7 percent waxy; his F_3 progenies, an average of 24.6 percent waxy. The difference between these two generations is 4.3 times the probable error and would be expected as the result of chance only once in about 267 trials.

The difference between the second and third generations cannot be explained by consistent errors in classification or to regular differences in viability, rate of pollen tube growth, or resistance to unfavorable influences inherent to the waxy factor *itself*, since all of these influences would produce as great a disturbance in one generation as in another. The situation, however, is exactly what would be expected if an accessory factor linked with the waxy gene is involved.

Further evidence of the action of an accessory factor influencing the waxy ratio is found in the data in table 1 of KEMPTON'S paper (1919). In this table are given all the results of selfing and intercrossing F_1 plants. Ordinarily, of course, these two types of pollination should give identical results but if an accessory factor is present in part of the plants, differences might well occur. KEMPTON, in intercrossing a pair of F_1 plants, frequently also self-pollinated one or both members of the pair and the data resulting from selfing are, fortunately, included in the table and can be compared to those obtained from the intercrossing.

A study of the pedigree numbers in this table show that there are 26 intercrosses in which the segregation of the two F_1 plants used in making the cross, has also been determined by selfing. Some of the F_1 plants gave almost normal ratios, others showed a deficiency of waxy seeds. Arbitrarily dividing the selfed plants into *normal* and *low*, using a deviation of twice the probable error as a basis, the intercrosses can be arranged into the following groups; *low* × *low*, *normal* × *low*, *low* × *normal*, and *normal* × *normal*.

Obviously, if the deviations in the selfed plants are merely due to chance, then grouping the intercrosses on the basis of the segregation in the selfed parental plants can have no effect on the average percentage of waxy in each group. If, however, the deviations are due to some condition inherent in the plants, it might be expected that *low* × *low* intercrosses would give different results than *normal* × *normal* pollinations. The results of grouping the intercrosses according to the behavior of both members of the parental pair are shown in table 13.

It is noted that the *low* by *normal* and *normal* by *normal* intercrosses are very closely alike, giving 25.1 and 25.4 percent of waxy seeds respectively. The *normal* by *low* and *low* by *low* intercrosses also resemble each other giving 22.9 and 22.6 percent of waxy seeds respectively. In other words, the groups of intercrosses in which pollen from *normal* plants was used gave *normal* ratios, the average for the two groups being 25.3 percent recessives, a very close approximation to the theoretical ratio. When pollen from *low* waxy plants was used, however, the intercrosses gave *low* waxy ratios, the average for the two groups being 22.7 percent waxy, a deviation from the theoretical ratio of 5.8 times the probable error.

It should be mentioned that in addition to the intercrosses shown in table 13 there are two involving plants with *high* waxy ratios. One of these, *normal* × *high*, gave 21.3 percent waxy, the other, *high* × *low*, 28.1 percent waxy. No conclusions regarding the significance or meaning of plus deviations can be drawn from these two ears.

It is apparent that the arbitrary grouping of the F_1 plants into *low* and *normal* on the basis of their deviations has actually divided them into classes which are inherently distinct. Plants giving *low* ratios when selfed give almost exactly the same ratios when their pollen is used on other plants. The remaining plants give ratios closely approximating *normal* whether selfed or intercrossed. In other words, *the random assortment of the male gametes at fertilization is disturbed in about half of the plants, and is relatively normal in the remainder.*

These facts can readily be explained by assuming that one of the parents of KEMPTON's hybrids was heterozygous for an accessory factor which affected the chances of the two kinds of gametes in accomplishing fertilization. Probably it was the waxy stock which brought in the accessory gene because deviations have been found in almost every case that waxy has been crossed with other stocks.

With the evidence of an accessory factor in mind it should be possible to isolate from such crosses as KEMPTON'S, some heterozygous waxy lines which regularly give normal ratios, some which give a majority of minus deviations and others a majority of plus deviations. We have no doubt that such lines will soon be reported and that crosses with other characters on the waxy chromosome will indicate the approximate location of the accessory factor. BRINK (1925) has, in fact, evidence from one of his crosses which indicates the presence of an accessory factor closely linked with the *I* factor, which is located on the waxy chromosome.

The gametophyte factor in the waxy stock probably disturbs the ratios by causing differential pollen tube growth as does the factor in the sugary and defective seeds stocks. At least this is the simplest explanation and there is some evidence of its correctness from BRINK'S (1925) experiments in which he found that fewer waxy seeds were produced on ears with long styles compared to those in which the styles were cut short.

PECULIAR RATIOS IN COLOR SEGREGATION

It is not improbable that COULTER'S aberrant segregation in a stock heterozygous for the *C* factor (COULTER 1925) is due to the same accessory factor which affects the waxy ratios, since *C* and *wx* are members of the same linkage group.

COULTER'S stock produced some progenies with marked minus deviations of white seeds, some with marked plus deviations, and others with normal ratios. He explains the deviations on the basis of linkage with a zygotic lethal factor and it is true that they can be fairly satisfactorily explained on this basis, although some of his *high* white ratios are higher than would be expected. Zygotic lethals, even with complete linkage, (repulsion phase) should cause a distortion of the ratio only from a 3 : 1 to a 2 : 1. A large proportion of COULTER'S high ears average higher than 33.3 percent recessives and a number of them show 38 to 40 percent of white seeds.

Furthermore, zygotic lethals in maize are usually expressed in the form of defective seeds, and when these are present they can be readily noted and their numbers accurately determined. COULTER asserts that aborted

seeds are sometimes found in this stock but apparently not regularly. He writes[1] "Many of my ears have the random distribution of empty pericarps so characteristic of the defectives. On other ears it seems impossible to detect the presence of the abortive grains. Doubtless accessory factors are at play to determine the degree of expression of this character."

From the experience in studying the inheritance of fourteen types of defective seeds, (MANGELSDORF 1926), it may be said that when defective seeds are present at all, their segregation is usually clear-cut and their number can be readily determined. The accessory factor which COULTER postulates is probably not one which so affects the degree of expression of the defectives that they can no longer be distinguished from the normal seeds but rather a gametophyte factor which influences the ratio in which the defective seeds appear, as well as the segregation for color. If this is true it should be possible to isolate from COULTER's stock, lines which give *high, normal* and *low* white progenies but which do not segregate for defective seeds, others which give *high, normal*, and *low* defective progenies but do not segregate for color.

HOW GAMETOPHYTE FACTORS OPERATE

If the gametophyte factor on the waxy chromosome is one which affects the rate of pollen tube growth, it differs in two respects from the *Ga* factor on the sugary-defective chromosome. The first difference is that the waxy pollen tube factors reduces the rate of pollen tube growth instead of stimulating it. The second difference is that it operates regardless of the genetic constitution of the sporophytic tissues in which the pollen tubes grow. This is shown by the fact that the *normal* × *low* intercrosses in table 13 gave exactly the same results as the *low* × *low*.

There is, of course, no reason for believing that all gametophyte factors behave in the same way. It is probable that factors operating in the gametophyte generation may have their effect in many different ways, for example; in causing differential pollen tube growth, duration of viability, resistance to drying out, excessive moisture, high and low temperature, etc. Even the factors affecting the rate of pollen tube growth alone may fall into at least four distinct categories, as follows:

1. Factors which reduce the rate of pollen tube growth only when the tubes are growing in sporophytic tissue of the same genetic constitution.

2. Factors which reduce the rate of pollen tube growth regardless of the genetic constitution of the sporophytic tissues.

[1] In a letter.

3. Factors which stimulate the rate of pollen tube growth only when the tubes are growing in sporophytic tissue of the same genetic constitution.

4. Factors which stimulate pollen tube growth regardless of the genetic constitution of the sporophytic tissues.

TABLE 13

Kempton's F_1 intercrosses grouped on the basis of the segregation in the selfed parental plants.

TYPE OF INTERCROSS	PEDIGREE NUMBERS			PERCENTAGE WAXY		
	♀	♂	INTERCROSS	♀	♂	INTERCROSS
L×L	1534	1743	1535	21.7	20.5	22.7
L×L	1543	1723	1542	20.9	21.0	23.3
L×L	1723	1543	1722	21.0	20.9	23.9
L×L	1743	1534	1742	20.5	21.7	21.3
Average						22.9
N×L	1117	1118	1116	24.2	21.9	26.0
N×L	1515	1726	1518	25.0	21.6	21.5
N×L	1129	1135	1130	24.1	21.5	21.0
N×L	1133	1118	1134	24.6	21.9	16.4
N×L	1137	1135	1138	26.0	21.5	25.1
N×L	1741	1551	1740	22.7	21.2	25.6
Average						22.6
L×N	1111	1132	1110	22.2	23.3	27.5
L×N	1118	1108	1119	21.9	26.4	25.8
L×N	1121	1117	1120	22.5	24.2	22.1
L×N	1551	1741	1550	21.2	22.7	20.6
L×N	1135	1137	1136	21.5	26.0	28.6
L×N	1726	1519	1725	21.6	23.8	24.2
Average						25.1
N×N	1105	1108	1104	26.4	26.4	23.7
N×N	1527	1745	1526	23.3	23.8	26.8
N×N	1549	1749	1548	24.6	26.3	24.0
N×N	1128	1125	1127	25.0	24.0	24.4
N×N	1132	1117	1131	23.3	24.2	22.9
N×N	1721	1515	1720	23.2	25.0	30.7
N×N	1745	1527	1744	23.8	23.3	26.0
N×N	1749	1549	1748	26.3	24.6	25.8
Average						25.4

Gametophyte factors which fall into the second and third of these categories have apparently already been found in maize in the waxy and sugary-defective pollen tube factors. An example of the first group is

found in the inheritance of self-sterile classes in Nicotiana (EAST and A. J. MANGELSDORF 1925, 1926) where certain classes are almost completely eliminated presumably because of the slower rate of pollen tube growth.

CARBOHYDRATE METABOLISM AND POLLEN TUBE GROWTH

It may have been noted that all the characters so far reported *su*, *wx*, de_1 and de_{su} which appear to be associated with differences in pollen tube growth, are endosperm characters which are also associated with differences in the nature or amount of carbohydrate storage.

BRINK (1925) is of the opinion that differential pollen tube growth operates in combination with, or is caused by, differential carbohydrate metabolism. The fact that these four above mentioned characters do affect the nature or amount of carbohydrate storage might be considered as evidence in favor of such a view. We are of the opinion, however, that this is merely a coincidence; that distorted ratios in maize have been found mainly in endosperm characters only because such characters have been studied on a larger scale than any others. We feel certain that similar disturbances in the ratios of seedling and plant characters will also be found when these are studied on a greater scale. In fact, *high*, *low*, and *normal* ratios, apparently due to differential pollen tube growth, are now being obtained in a white seedling stock. (MANGELSDORF Unpublished).

The possibility, however, that differential carbohydrate metabolism may have some effect on pollen tube growth and that certain sporophytic characters may *per se* affect the rate of growth, is not denied. The marked deficiency of waxy seeds which BRINK (1925) obtained in a heterozygous waxy, homozygous sugary stock cannot, perhaps, be explained on the basis of gametophyte factors alone.

DISCUSSION

Selective fertilization has always offered an attractive explanation of peculiar results for which it was difficult to account by other means, and has frequently been called into account for unexpected departures from Mendelian ratios. Cuenot's yellow mice, CORREN'S aberrant ratios in Melandrium, HERIBERT-NILLSON'S unusual segregation of certain characters in *Oenothera*, are examples[1].

EAST (1922) includes as one of the "Provisional Laws of Heredity" the statement that "there is no selective fertilization between complementary, compatible, functional gametes". This is probably true if the term

[1] BRIEGER (1926) has recently summarized the cases in which selective fertilization might be used to interpret the results.

"fertilization" is confined to the actual fusion of the nuclei and a distinction is made between selective fertilization and certain other influences which disturb the random assortment of the gametes at the time of fertilization. For example; in plants or animals in which free-swimming, motile sperm come in contact with the egg in large numbers, there may be no discrimination by the female gametes in favor of certain of the male gametes, providing that all are compatible. There is no reason for believing, however, that all the sperm, in all cases, are equally capable of reaching the egg. It is possible that some classes are so handicapped as a result of their chromosomal constitution that though produced in full quota at gametogenesis, they are in minority at the time of fertilization.

In angiosperms an entire generation elapses between the time of gametogenesis and fertilization and the opportunity for differential elimination of certain classes is relatively large.

The pollen tube of angiosperms represents the remnant of the elaborate gametophyte generation in lower plants, in many of which this generation is of equal or greater importance than the sporophyte. In such plants there can be no question that the hereditary factors have almost, if not quite, as great an influence in the gametophyte as in the sporophyte generation. In fact, characters which have their expression in the gametophyte generation have recently been reported by WETTSTEIN (1924) in the mosses and ALLEN (1925) in the liverworts. In angiosperms the gametophyte generation has become greatly reduced and the hereditary factors which influence it have, no doubt, been correspondingly lessened, though not entirely eliminated.

Mendelian factors have been found to affect practically all stages in the ontogeny of the maize plant. The growing embryo, the endosperm, the resting stage of the seed, the young seedling, stature, chlorophyll development and reproductive processes, are all governed by genetic factors which may be unlike for different individuals. *It would be strange indeed if the gametophyte generation, brief though it has become, should be entirely deprived of the governing influence of various Mendelian factors.*

It is not surprising, therefore, to find in maize several Mendelian factors which have their expression in the gametophyte generation. These factors produce a definite physiological effect upon the gametophytes in which they occur, speeding up development in one case, reducing it in the other. They are transmitted from generation to generation in the same manner as any other Mendelian factors, occupy definite loci on certain chromosomes and show characteristic linkage and crossing over phenomena.

In general, the genetic factors which affect the gametophyte probably have little influence on ordinary Mendelian ratios in the sporophyte. In the female gametophyte, at least in angiosperms, they probably seldom cause any disturbance. In the male gametophytes they can have no effect as long as the parental sporophyte remains homozygous for the factors which influence the gemetophyte generation. Even when the gametophytes differ genetically, the favorable and unfavorable factors would, in the long run, tend to balance each other and no classes of gametes would be given a decided advantage. Only in occasional instances where a gametophyte factor produces a marked effect and is at the same time closely linked with a recognizable character in the sporophyte, is there any disturbance in the Mendelian ratios. Even here the total disturbance may not be noticeable when data from several generations are combined because a reversal of the linkage relations will cause deviations to balance each other.

Genetic factors affecting the gametophyte generation, giving certain gametes an advantage or handicap in accomplishing fertilization, need not contradict the Mendelian conception of random assortment. In some plants, however, they must be taken into consideration, as are linkage and lethal factors, in accounting for unexpected results.

SUMMARY

1. A stock segregating for defective seeds, de_1, regularly produces *high* ears with approximately 33 percent recessives, *normal* ears with 25 percent recessives, and *low* ears with 17 percent recessives.

2. These results are explained by assuming a factor, *Ga*, which has its expression in the gametophyte generation, stimulating the rate of pollen tube growth. *Ga* is linked with de_1 and in the coupling phase causes an excess of recessives; in the repulsion phase, a deficiency.

3. The cross of sugary × Rice Pop indicates the presence of a similar gametophyte factor linked with *Su* in the Rice Pop parent. The percentage of sugary seeds in F_2 is 16.2.

4. *Su* and de_1 are linked, with 39 percent crossing over, and it is probable that the gametophyte factor in both stocks is the same.

5. On this assumption it has been calculated that the *Ga* gametes accomplish fertilization 4.1 times as frequently as *ga* gametes and that the crossing over between *Ga* and de_1 is 24.3 percent; between *Ga* and *Su*, 21.2 percent.

6. That these values are approximately correct is indicated by WENTZ' data from a cross between de_{su}, a fourth character in this group, and a

stock with Rice Pop ancestry. This cross resulted in deviations approaching those predicted by the above calculations.

7. Gametophyte factors probably account, to a large extent, for the peculiar results obtained in mixed pollination experiments.

8. An analysis of KEMPTON'S data indicates the presence, on the waxy chromosome, of a gametophyte factor causing distortions in the starchy: waxy ratio.

9. The action of the same factor is suggested as explanation of COULTER'S peculiar ratios in aleurone color segregation.

10. The various ways in which gametophyte factors may operate and their effects on Mendelian ratios in the sporophyte are discussed in detail.

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UNIT CHARACTER INHERITANCE OF COLOR IN THE BLACK RAT, *MUS RATTUS* L

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INTRODUCTORY REMARKS

A presentation of certain facts concerning the inheritance of color in the Black rat, *Mus rattus rattus* L., and its allies is justified for at least two reasons. The inheritance of color variations in these rodents illustrates the type which may exist in essentially wild mammals. The extent of knowledge of the inheritance of similar variations in other rodents makes interesting comparisons possible.

The literature pertaining to the old-world rats in earlier times is not altogether clear. *Mus norvegicus* Erxleben, or *Mus decumanus* Pallas, commonly termed the Norway or brown rat, seems to have been confused with the members of the *rattus* group by several writers in spite of its distinctive characters and history. The *Mus rattus* group includes a considerable series of forms which are described at some length by HINTON (1919). As a group they are probably indigenous to INDIA. Extension of their range into Europe is evidenced by remains in pliocene deposits in LOMBARDY, in the quaternary near PISA, in pleistocene deposits on the island of CRETE, and in glacial times and with remains of the lake dwellers in western GERMANY and MECKLENBURG. At the present time the largest variety of forms occurs in southern ASIA. Ships have conveyed members of the group, however, to practically all parts. In the UNITED STATES well established colonies of the black rat, *Mus rattus rattus*, occur in northern localities, especially NEW ENGLAND. The roof rat, *M. rattus alexandrinus*, Geoffroy, is common in the southern states, especially along the seaboard.

Specimens of *M. rattus*, taken wild, exhibit a considerable range of color variability. The typical black rat on the one hand, is essentially black, while the roof rat is agouti dorsally, and creamy white ventrally. The variability within each of the types is also great. Certain subspecies and races from northern AFRICA, INDIA and CHINA are similar to the roof rat dorsally but show ventral pigmentation of a dusky or gray type. Such races resemble the Norway rat in coloration. Geographical isolation supplemented by mutation seems to have produced at least three distinct types in several localities.

MORGAN (1909) crossed the black rat with the roof rat. All of the resulting 32 animals were black. These mated *inter se* produced 4 black and 1 gray progeny. It was concluded that black is dominant over gray or agouti.

More recently several additional color variations have been reported which evidently occurred as mutations in wild colonies. PATTERSON (1920) described a cinnamon type which constituted approximately 7.5 percent of a population of the roof rat on two adjacent farms in TRAVIS COUNTY, TEXAS. HAGEDOORN and HAGEDOORN (1922) noted several variations in a hybrid population from several subspecies. Yellow, chocolate, waltzing, white tail-tip, and albinism were included.

CREW (1923) described a mutant of the black rat which occurred in warehouses of BRISTOL, ENGLAND in 1920. This form had a fawn and white coat and black eyes. It behaved as a Mendelian recessive character in crosses with the black. CREW stated that it was not the same form which HAGEDOORN described, for it did not have red eyes which darkened with age, as did their yellow type.

The writer (FELDMAN 1923) described the inheritance behavior of a black variety which was recessive or hypostatic to agouti. Also, in 1923 (FELDMAN 1923a) the knowledge of color inheritance in *Mus rattus rattus* and *M. rattus alexandrinus* was presented in a summarized form as far as the experiments had revealed at that time.

The purpose of this paper is to describe briefly the appearance of certain color varieties of these rats and to give the mode of their inheritance which the experiments have thus far indicated. They include black varieties (epistatic and hypostatic to agouti), agouti with white belly, agouti with gray or ticked belly, cream dilution and "blue" dilution. The nomenclature used in the descriptions is based on that of RIDGWAY (1912). Careful comparisons of the rats with his color standards were made in the daylight. The experimental evidence on the inheritance of the variations

was gained through records of 943 individuals obtained in a series of different, controlled matings from 1922 to 1925 inclusive.

Opportunity is taken at this point to thank Doctors C. C. LITTLE and W. H. GATES for supplying live individuals of the black rat and roof rat respectively. The writer is indebted to Doctor W. E. CASTLE for his kindness in making the entire stock of the species available for investigation and for his helpful suggestions at various times.

DESCRIPTIONS OF THE VARIETIES

The original specimens of the black rat, *Mus rattus rattus*, were obtained from Doctor C. C. LITTLE who received them from Mr. P. C. HEALD of WILTON, NEW HAMPSHIRE. The four males and one female were very similar in appearance. Three of the males were successfully bred. The coloration of the black rat is typical of melanic forms. The mid-dorsal region is black and in some specimens is iridescent. The sides gradually shade from blackish mouse-gray to mouse-gray on the ventral surface. The six shades of neutral gray from dusky to pale also describe the color of this form, but slightly less accurately. On the sides and ventral surface of most individuals are scattered light hairs of cartridge buff. The skin of the ears, tail and feet is dusky mouse-gray. The eyes are black.

Typical roof rats, *Mus rattus alexandrinus*, were sent to us by Doctor W. H. GATES from BATON ROUGE, LOUISIANA. They consisted of two young females which produced numerous progenies. This form has a typical agouti pigmentation. With the exception of the ventral surface, the pelage is composed mainly of hairs which are dark to light mouse-gray at the base and which are tipped with buff varying from ochraceous to pale ochraceous. Among the parti-colored hairs, completely dark and dark-tipped ones are scattered. The intensity of the black and yellow pigments is greatest on the mid-dorsal area, and becomes gradually lighter as the cartridge buff to white ventral surface is approached. This light area which comprises approximately one-fourth of the total, is sharply defined. The skin on the ears, tail and feet is mouse-gray in color and is thinly covered with buff-colored hair. The eyes are black.

In a shipment of rats of the *rattus* group received from Mr. H. C. BROOKE, a fancier of TAUNTON, ENGLAND, two additional color types were apparent and experiments revealed still another. Of three agouti females, two were typical of the roof rat coloration, agouti with light belly. The third, however, had a dark or ticked ventral coloring which resembled that of the Norway rat. This type reoccurred in the descendants of the female

and other rats of the shipment. The dorsal and lateral coloration of the variety agouti with dark belly, is similar to the typical roof rat. The average individual is slightly darker, however, due to a larger proportion of hairs which are either entirely dark or are dark at the tip as well as at the base. The ventral hairs have a base of mouse-gray and an apex of cartridge buff. The skin at the extremities is very similar to that of the black rat. The eyes are also black. In this variety we evidently have the same coloration which is found in colonies of the wild subspecies.

The second new color type in the shipment from Mr. BROOKE was a cream variety. The coat in this form is typically cartridge buff to ochraceous buff on the mid-dorsal region, becoming lighter on the sides. The ventral surface is about six shades lighter than the mid-dorsal and is usually creamy-white. Approximately half of the cream animals have dorsal areas in which some of the hairs have light mouse-gray bases. A sooty appearance results. There is considerable individual variability in the intensity of the yellow and the intensity and extent of the sooty areas. The skin on the ears, tail, and feet is pallid mouse-gray colored. The eyes are black. This type is undoubtedly the one described by CREW, *loc. cit.*, and named by him *Mus rattus rattus* var. *brookei*, since our stock came from the same source as CREW's.

There appeared in matings between agouti individuals and between agouti and cream, a black type. Breeding tests demonstrated that this is a new black variety which resembles the typical black rat in appearance, but has a different genetic relation to the other varieties.

In September, 1923, we received from Mr. BROOKE a "blue" variety. He has informed us that 4 "blue", 1 "blue-gray" and a number of black specimens were trapped on a boat at LIVERPOOL, ENGLAND, in the spring of 1923. The three males and two females which were sent to us were offspring of these animals. The color of this variety is dark mouse-gray dorsally shading to pallid mouse-gray on the ventral side. The skin at the extremities is mouse-gray. The eyes are black.

A survey of what is known regarding the origin of these types of *Mus rattus* favors the view that the agouti pigmentation with white belly which is characteristic of the roof rat is the primitive type. The melanism characteristic of the black rat, and the type which is gray with dark belly probably arose as mutations at some time sufficiently remote to give rise to subspecies. The second (recessive) black type occurred as a mutation in the roof rat in recent times. While the cream, *M. rattus rattus* var. *brookei*, and the "blue" variety are recent mutations of the black rat.

The five different types of these rats are represented in figure 1.

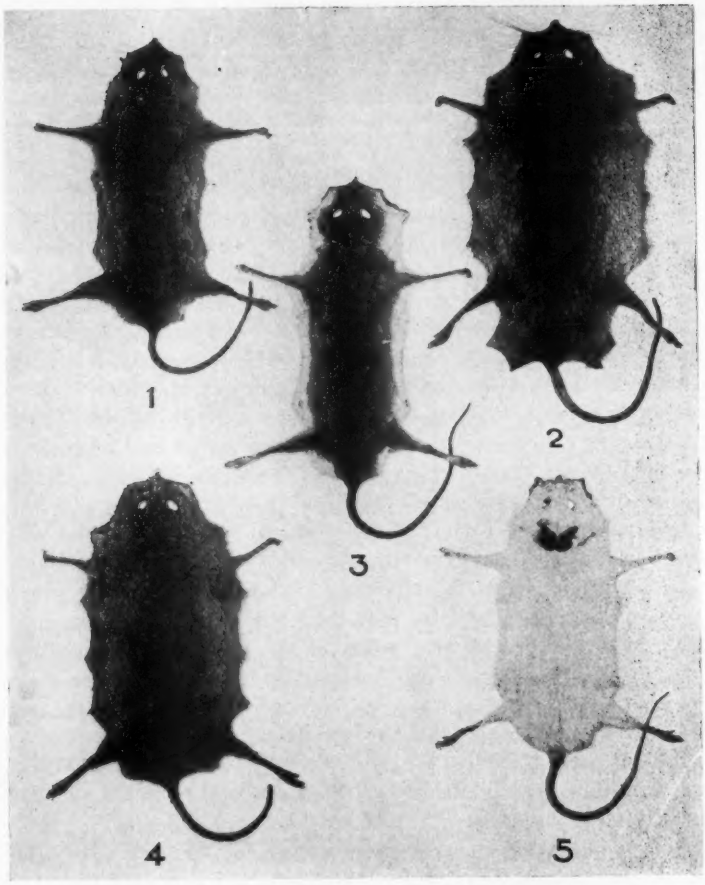

Figure 1. Skins representing five color types of, *Mus rattus*.

1. Typical Black rat, *Mus rattus rattus*.
2. Agouti variety with ticked ventral coloring.
3. Typical Roof rat, *Mus rattus alexandrinus*.
4. Dilute or "blue" variety.
5. Cream variety, *Mus rattus rattus* var. *brookei*.

GENETIC RELATIONSHIPS OF THE VARIETIES

The experimental data indicate a simple Mendelian relationship of the factors producing the color varieties of this species. Thus far no indication of the linkage of any of the factors has been observed, although specific tests for it have not been made. The pertinent data are included in table 1.

TABLE 1

Summary of data on the inheritance of color in the Black Rat, Mus rattus rattus, and its allies.

NUMBER OF MATING	COLOR TYPES OF PARENTS	COLOR TYPES OF OFFSPRING					
		Black	Gray wh. ve.	Gray d. ve.	Cream	Blue	Total
1.	Gray (white ventrally) *inter se*	5	12	1	9	..	27
2.	Gray (white ventrally)× gray(dark ventrally)	1	11	2	1	..	15
3.	Gray (white ventrally)× black(recessive)	3	20	..	2	..	25
4.	Gray (dark ventrally) *inter se*	5	..	13	3	..	21
5.	Gray (dark ventrally)× black (recessive)	13	..	17	2	..	32
6.	Black (recessive) *inter se*	18	..	..	3	..	21
7.	Gray (white ventrally)× cream	11	21	1	25	..	58
8.	Gray (dark ventrally)× cream	4	8	5	8	..	25
9.	Black (recessive)×cream	2	1	..	11	..	14
10.	Cream *inter se*	..	..	..	246	..	246
11.	Black (dominant)× Gray (white ventrally)	42	..	..	..	..	42
12.	F_1 from mating 11 *inter se*	46	16	..	..	..	62
13.	Black (dominant)×cream	36	..	..	..	..	36
14.	F_1 from mating 13 *inter se*	124	..	..	45	..	169
15.	F_1 from mating 11× black (recessive)	18	7	..	..	..	25
16.	F_1 from mating 13×cream	4	..	..	4	..	8
17.	Blue×blue	..	..	..	..	12	12
18.	Blue×gray (white ventrally)	7	..	..	..	..	7
19.	Blue×black (recessive)	14	..	..	..	..	14
20.	Blue×cream	17	..	..	..	..	17
21.	F_1 from mating 20 *inter se*	7	..	..	4	2	13

Three allelomorphs of the black extension factor exist. They are the melanism characteristic of the black rat, ordinary extension characteristic of the agouti pigmentation of the roof rat, and practical absence of black in the coat which exists in the cream or yellow variety. They may be represented by the symbols, E', E, and e respectively. Ordinary extension is epistatic to cream and the black is epistatic to both. Wild caught black rats, $E'E'$, mated to agouti roof rats, EE, produced black F_1, $E'E$ (see

mating 11 in the table). The F_1 mated *inter se* produced approximately 3 black E': 1 agouti, E, young (mating 12). Likewise, the black rats, $E'E'$, mated to creams, ee, which were known to carry the agouti factor, produced all black, $E'e$, in the F_1 generation (mating 13). These F_1 mated *inter se* produced approximately 3 black E',: 1 cream, e, but no agouti colored, E, individuals (mating 14). When an F_1 male, $E'e$, was mated to a cream female, ee, a litter of 4 black and 4 cream young was produced. Rats which possessed ordinary extension, E, mated *inter se* produced a total of 141 offspring of which 20 were cream (matings 1 to 6). The deviation is accounted for by the fact that some of them were homozygous, EE. Animals of ordinary extension when crossed with creams produced 53 ordinary extension, Ee,: 44 creams, ee (matings 7 to 9). Again, the deviation from equality in this case was probably due to the homozygosity of some of the rats, EE. Creams mated to creams produced 246 young, all of which were cream (mating 10).

Individuals which are phenotypically ordinary extension, that is, of the composition, EE, or Ee, may show any one of three allelomorphic conditions of the agouti factor. These allelomorphs are, agouti with white belly, A^w, agouti with dark belly, A^d, and non-agouti, a. There is complete dominance of A^w, over A^d and a, and of A^d over a. The matings between these types may be seen to corroborate these statements (matings 1 to 6). A general excess of the dominant types is explicable by assuming the homozygosity of some of the animals involved for either A^w or A^d. Segregation of the recessive type or types was thus prevented in their particular litters. The E types when crossed with e types showed that the latter could possess any of the agouti factors in either a homozygous or heterozygous state.

The matings involving the "blue" dilution character, d, are very incomplete as yet. They demonstrate that it is recessive to normal intensity. They also show that the original "blue" animals were homozygous for the black extension factor, E'. All F_1 animals from matings between "blues" and agouti with white belly, recessive black, and cream rats were black, (matings 18, 19, 20). The observations of Mr. BROOKE referred to above indicate that the "blue" may occur in conjunction with agouti in the same individual, however. The matings show further that "blue" dilution is not an allelomorph of either the black extension series or of the agouti series. Crosses between "blue" and recessive members of both series produce in F_1 a reversion to the dominant type. Rats of two litters produced by mating the F_1 from the "blue" $\times$ cream cross *inter se* consisted of 7 black, 4 cream and 2 "blue" individuals. Two of the cream rats in these litters were

double recessive *ee dd* in nature. "Blue" appears to segregate, therefore as a typical recessive Mendelian character. "Blue" rats mated *inter se* produce only "blue" offspring.

With this survey of the genetics of color in these rats it is possible to state the factorial formulae of the animals which were used as follows:

1. Wild black rats, *E'E' DD*. (Their composition for the *A* factor has not been determined).[1]
2. Wild roof rats, *EE A^wA^w DD*.
3. Agouti rats with white belly, *EE A^wA^w DD*, *Ee A^wA^w DD*, *EE A^wA^d DD*, *Ee A^wA^d DD*, *EE A^wa DD*, and *Ee A^wa DD*.
4. Agouti rats with dark belly, *EEA^dA^dDD*, *EeA^dA^dDD*, *EEA^daDD*, and *EeA^daDD*.
5. Recessive black or non-agouti rats, *EE aa DD*, and *Ee aa DD*.
6. Cream rats, *ee A^wA^w DD*, *ee A^wA^d DD*, *ee A^wa DD*, *ee A^dA^d DD*, *ee A^da DD*, and *ee aa DD*.
7. "Blue" rats, *E'E' dd*. (Their composition for the *A* factor has not been determined.[1]

DISCUSSION

The genetics of the color varieties of *Mus rattus*, shows that two loci of the germ-plasm have undergone two changes or mutations each to produce the black extension series and the agouti series of allelomorphs. A third locus has mutated once to produce "blue" dilution. Three different chromosomes seem to be involved. In all probability additional loci have produced the cinnamon, chocolate and albino varieties described by other investigators. At least two of these characters which mendelize, namely epistatic black and agouti with dark belly are characters which distinguish subspecies.

The unit characters of the black rat and its allies with which this paper deals are not unknown in other forms, especially in other laboratory rodents. There are varieties of the Norway rat, house mouse, guinea-pig and rabbit which resemble them very closely in appearance and hereditary behavior. It is unsafe, however, to assume that the characters of the black rat have exact homologues in the other forms.

Black extension allelomorphs occur in the guinea-pig and rabbit. In both species ordinary extension *E*, present in the wild agouti types, has

[1] Since this paper was sent to press, F_2 individuals from matings of dominant black with recessive black, and "blue" with recessive black have been secured. The occurrence and proportions of agouti individuals with gray or ticked belly coloring indicate that the dominant black and "blue" rats carry that allelomorph of the agouti factor. They are, therefore, of the factorial composition, *E'E' A^dA^d DD* and *E'E' A^dA^d dd* respectively.

two recessive allelomorphs. Tortoise-shell in the guinea-pig and the Japanese rabbit are mosaics of yellow and black which may be designated as e^t. They are recessive to E and dominant over yellow coat-color, e. In the rabbit a fourth allelomorph, epistatic black, E', exists. It is apparent that the series in *Mus rattus* has three allelomorphs in common with the rabbit, namely epistatic black, E', agouti extension, E, and cream, e. It also has the latter two in common with the guinea-pig. The mutation corresponding to the tortoise-shell and Japanese varieties has not been observed in this rat.

TABLE 2

Summary of allelomorphs of the black extension factor in the laboratory rodents.

	E'	E	e^t	e
Rabbit	X	X	X	X
Guinea-pig	..	X	X	X
Norway rat	..	X	..	..
Black rat	X	X	..	X
House mouse	..	X	..	..

The agouti allelomorph series of the Black rat also shows considerable parallelism to that of the other rodents. The non-agouti mutation a, has been observed in the rabbit, guinea-pig, Norway rat, and house mouse. The guinea-pig, Norway rat and house mouse also exhibit agouti with dark ventral pigmentation, the third allelemorph of the Black rat. In the Norway rat the wild form has this type of agouti. A species of cavy, *Cavia rufescens*, studied by DETLEFSEN (1914) also shows this type of agouti in the wild state. The house mouse has in addition to the two types of agouti and non-agouti, an epistatic yellow allelomorph which has no counterpart in the series of other rodents. The black-and-tan variety of the rabbit, also an agouti allelomorph, has no counterpart among other forms. The three allelomorphic conditions of the agouti factor, A^w, A^d, and a of the Black rat are all present, therefore, in the guinea-pig and house mouse with the same order of dominance. Agouti with white belly, A^w, is lacking in the Norway rat, and the rabbit has no character corresponding to the agouti with dark belly, A^d.

TABLE 3

Summary of allelomorphs of the agouti factor in the laboratory rodents.

	A^y	A^w	A^d	a^t	a
Rabbit	..	X	..	X	X
Guinea-pig	..	X	X	..	X
Norway rat	..	..	X	..	X
Black rat	..	X	X	..	X
House mouse	X	X	X	..	X

As in the case of the other color characters of *Mus rattus*, "blue" dilution also occurs in other species. "Blue" varieties of the rabbit and house mouse are known which are Mendelian recessive characters to "normal" intensity. The factors producing the color varieties in these rodents indicate strongly the tendency of similar mutations or germinal changes to occur in closely related species. It is further evidence of a constant tendency of certain loci to mutate more frequently than others in any given species.

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STUDIES ON SELF-STERILITY VII. HEREDITY AND SELECTIVE POLLEN-TUBE GROWTH

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INTRODUCTION

A new genetic interpretation of self-sterility as manifested in *Nicotiana alata* Lk. and Otto *var. grandiflora* Comes and *Nicotiana forgetiana* Hort. (Sand.), or their hybrids, has been proposed recently (EAST and MANGELSDORF 1925). The detailed evidence on which this interpretation rests is given in the present paper.

Self-sterility appears to behave as a simple recessive when these species are crossed with the self-fertile species, *Nicotiana Langsdorffii* L. Their interaction is thus similar to that found by COMPTON (1912) in crosses between self-fertile and self-sterile strains of *Reseda odorata* L. The strains of Nicotiana homozygous for self-sterility behave as if their peculiar condition were controlled by several hereditary factors. Like combinations of factors produce like groups of plants in so far as their compatibility with each other is concerned, just as like factorial combinations which lie at the basis of morphological differences produce groups which are alike in those characters. Generally speaking, all members of a given self-sterility group are cross-sterile with each other reciprocally, but are cross-fertile with the members of every other group.

These distinctions in compatibility are due to hereditarily controlled reactions of such a nature that some pollen-tubes (compatible matings) show a rapidly accelerated rate of growth which permits fertilization in from three to five days,—depending mainly on the temperature, while other pollen-tubes (incompatible matings) show a slower and more nearly uniform rate of growth which ordinarily does not allow the generative nucleus to reach the micropyle before the flower withers and falls.

Obviously the differences between compatible and incompatible matings are quantitative, as were the differences between Mendel's tall and dwarf Pisum plants; nevertheless these differences are definite and concrete. There is no more reason for confusing the two types, as certain writers have done, than there is for confusing allelomorphs of numerous well demonstrated heritable differences of the kind commonly termed qualitative. For example, there are species of plants which produce colored flowers under ordinary horticultural conditions; but these plants may be carried through to the flowering stage under conditions which prevent the development of color in the petals, thus they will appear to belong to the same class as plants of the same species which lack the hereditary factor or factors necessary for color development and for which no yet discovered set of environmental conditions can promote color development.

Pollen-tube growth has been studied carefully in both compatible and incompatible matings of these self-sterile Nicotianas. Their growth-curves are characteristically distinct, and under the conditions most frequently met they do not overlap. In every slide studied a few pollen grains do not germinate, or, if they germinate, barely push through the stigmatic tissue. From the pedigree culture results on compatible matings wherein there is no deficiency of expected classes, we feel inclined to believe that this phenomenon is wholly somatic, unconnected with genetic differences. The remaining pollen-tubes in compatible unions show growth-curves having a very narrow variability; that is to say, fertilization in compatible matings is effected between the third and fourth day under all ordinary conditions, and there is probably not more than two hours' difference between the major and minor extremes. Furthermore variations in the environmental factors, such as moisture, plant age, etc., appear to advance or retard pollen-tube growth in these cases but slightly. Here only temperature changes seem to have an influence. In incompatible matings, on the other hand, external conditions have a marked effect. At the height of the flowering season, when pollinations are made on newly opened flowers, the average pollen-tube grows only about 8 mm in the length of time required by the pollen-tubes in compatible matings to traverse the whole length of the style (30 mm to 40 mm). It is noticeable, also, that the dispersion is much greater in the frequency distributions of incompatible matings than it is in compatible matings, though no precise determinations have yet been made. The rate of pollen-tube growth is especially affected by temperature variations and by the relative length of day and night, though it is difficult to determine the exact effect of each factor. One may say with some degree of confidence, however, that with vigorous plants, optimum

temperature and light conditions will not affect mid-season incompatible matings sufficiently to produce confused results, though occasionally a small seed-capsule can be obtained.

Fortunately for the analysis of these sterility phenomena, there is another means of obtaining relatively well-filled capsules of seed from incompatible matings. The time during which pollen-tube growth can occur may be materially prolonged. Normally, at mid-season, the individual flower remains on the plant from 6 to 7 days if fertilization does not occur in the meantime. Toward the end of the flowering season this period, the flower life becomes longer. During the last two weeks of flowering the flowers remain alive 9 or 10 days, while one instance is recorded of 14 days.[1] Time of pollen-tube growth can also be extended by bud-pollinations. The old idea that stigmas are only receptive during a limited period, and particularly when they are secreting a substance assumed to promote pollen germination, certainly must have a limited application. In numerous species we have found that pollination of the young bud is fully as efficacious as pollination of the mature flower. In fact it may be that the secretions found in many so-called "ripe" stigmas are merely manifestations of cellular decomposition which occurs after the proper time for pollination. At any rate pollinations can be made 3 days before the flowers open in the species we are considering without any evidence of abnormal effects. In fact pollen-tube growth apparently is faster in bud pollinations than it is in end-season pollinations of open flowers. At least, incompatible matings made on buds 2 days before they open often yield full complements of seed, while similar matings made on newly opened end-season flowers which remain on the plants 3 days over the average, seldom yield more than one-half the normal amount of seed.

By taking advantage of this second means of ensuring fertilization after incompatible matings, inbred strains of our self-sterile hybrids between *N. alata grandiflora* and *N. forgetiana* have been carried through fourteen generations. The purpose of this procedure was to eliminate heterozygotes in case the factors controlling the behavior of self-sterile plants should be distributed in the ordinary manner at maturation. Should compatibility between self-sterile plants be brought about by the existence of constitutional differences in certain factors, then one ought to obtain near-homozygous populations after successive selfings or close matings which would consist of very few intra-sterile, inter-fertile classes. This, in fact, has been the case. Several families have been raised in which the plants all belonged

[1] SLATE, working in this laboratory, has determined recently that these times may be much greater in certain families.

to two intra-sterile groups, while a few families have been found to consist of only one such group. The purpose of this paper is to describe the reactions of these families.

PRESENTATION OF DATA

As soon as several families were obtained which consisted of but two intra-sterile classes, all possible combinations were made between them. Peculiar but consistent results were obtained. These results can be illustrated by calling the classes X, Y and Z. X crossed by Y gave classes Y and Z in approximately equal numbers. The reciprocal cross Y with X gave classes X and Z in about the same proportions. The same phenomena appeared when the other combinations were made. Class X crossed with class Z gave approximate equality of classes Y and Z, but class Z pollinated with pollen from plants of class X gave equal numbers of classes X and Y. Similarly class Y by class Z produced only X's and Z's in equal numbers, while class Z by class Y produced X's and Y's in equal numbers.

Thus it appeared that when individuals from any two classes were crossed, *two* equal sized classes resulted, but the class to which the female parent belonged was never represented.

These results may be interpreted by assuming that the cross-sterility here shown is controlled by three allelomorphs, S_1, S_2 and S_3; and that class X is S_1S_3, class Y is S_1S_2 and class Z is S_2S_3. If then it is further assumed that a plant affords stimulus only to pollen bearing sterility factors other than its own, all the data are satisfied.

When a Z (S_2S_3) female is crossed with an X (S_1S_3) male only the pollen bearing the factor S_1 is stimulated and functions. Segregation is of the ordinary type, but differential pollen-tube growth causes fertilization to be selective. The resulting progeny, therefore, consists only of the two classes S_1S_2 (Class Y) and S_1S_3 (Class X) in equal numbers. In the reciprocal cross, X (S_1S_3) female by Z (S_2S_3) male, the X female again affords stimulus only to gametes bearing factors other than its own, but this time it is the S_2 pollen. The resulting progeny is therefore solely of the two classes Y (S_1S_2) and Z (S_2S_3). Data from matings of this type are presented in tables 1 and 2.

Six crosses were made where the female parents had the constitution S_2S_3(Z) and the male parents had the constitution S_1S_3(X). Of the resulting plants 405 were classified by crossing with X, Y and Z testers. They fell into two classes; 201 were S_1S_2 while 204 were S_1S_3.

The reciprocal cross, S_1S_3 by S_2S_3, was made seven times. The progeny again fell into two classes, this time S_1S_2 of which there were 233, and S_2S_3 of which there were 205, a total of 438.

TABLE 1

Results of crossing Class S_2S_3 female with Class S_1S_3 male.

PARENTAGE	PROGENY		TOTAL
	S_1S_3	S_2S_3	
L1×8 *L1Q2*	52	59	111
L1×22 *L1Q2*	26	25	51
L1×23 *L1Q2*	39	43	82
L1×7 *R1L1*	56	47	103
L1×*Q1*	12	8	20
L1×*O2*	16	22	38
Total	201	204	405

Most probable expectancy, 202.5 : 202.5 Deviation, 1.5 P. E. 6.8

TABLE 2

Results of crossing Class S_1S_3 female with Class S_2S_3 male.

PARENTAGE	PROGENY		TOTAL
	S_1S_2	S_2S_3	
8 *L1Q2*×*L1*	56	50	106
22 *L1Q2*×*L1*	39	40	79
23 *L1Q2*×*L1*	46	46	92
7 *R1L1*×*L1*	43	19	62
Q1×*L1*	21	18	39
O2×*L1*	8	16	24
R3×*C13*	20	16	36
Total	233	205	438

Most probable expectancy, 219 : 219 Deviation, 14 P. E. 7.1

In a second set of experiments, classes Z (S_2S_3) and Y (S_1S_2) were tested. Nine matings were made where S_2S_3 individuals were used as females and S_1S_2 individuals as males. From the resulting population, 493 individuals were tested, of which 248 belonged to the group S_1S_2 and 245 to the group S_1S_3. Ten reciprocal matings were made, S_1S_2 individuals being used as females and S_2S_3 individuals as males. Tests were made on 575 individuals coming from these crosses. Again there were two groups only—312 of class S_1S_3 and 263 of class S_2S_3. Here was found the greatest deviation from equality, a deviation slightly more than 3 times the probable error. The data are set forth in tables 3 and 4.

TABLE 3

Results of crossing Class S_2S_3 female with Class S_1S_2 male.

PARENTAGE	PROGENY		TOTAL
	S_1S_2	S_1S_3	
*L*1×10201	44	41	85
*L*1×*E*2	45	40	85
*L*1×1301	26	17	43
*L*1×21*R*1*E*2	23	21	44
*L*1×28*L*1*Q*2	42	58	100
8*R*3*R*1×*R*1	28	12	40
*C*13×*E*2	10	10	20
*L*1×*O*1	13	23	36
*L*1×*Q*2	17	23	40
Total	248	245	493

Most probable expectancy 246.5 : 246.5 Deviation, 1.5 P. E. 7.5

TABLE 4

Results of crossing Class S_1S_2 female with Class S_2S_3 male.

PARENTAGE	PROGENY		TOTAL
	S_1S_2	S_2S_3	
10201×*L*1	58	54	112
*E*2×*L*1	39	28	67
1301×*L*1	23	14	37
21*R*1*E*2×*L*1	22	22	44
28*L*1*Q*2×*L*1	56	35	91
*R*1×8*R*3*R*1	25	28	53
*N*1×*L*1	11	8	19
*Q*2×*L*1	50	30	80
*R*1×*C*13	12	20	32
*R*1×*L*1	16	24	40
Total	312	263	575

Most probable expectancy 287.5 : 287.5. Deviation 24.5. P. E. 8.1

In a third series of crosses, plants having constitutions S_1S_2(Y) and S_1S_3(X) were tested. Seven matings were made with S_1S_2 plants as females and S_1S_3 plants as males. From among the progeny, 396 individuals were classified; 189 belonged to group S_1S_3 and 207 to group S_2S_3. Six reciprocal matings were made, S_1S_3 plants being used as females and S_1S_2 plants as males. Testing out 317 of the resulting plants, it was found that 169 belonged to group S_1S_2 and 148 to group S_2S_3. The data for these crosses are found in tables 5 and 6.

TABLE 5

Results of crossing Class S_1S_2 female with Class S_1S_3 male.

PARENTAGE	PROGENY		TOTAL
	S_1S_3	S_2S_3	
R1×22 *L1Q2*	45	41	86
R1×23 *L1Q2*	38	51	89
R1×39 *R1L1*	30	43	73
R1×*R3*	14	26	40
E2×*R3*	21	9	30
O1×*O2*	23	17	40
Q2×*Q1*	18	20	38
Total	189	207	396

Most probable expectancy 198 : 198. Deviation, 9. P. E. 6 7

TABLE 6

Results of crossing Class S_1S_2 female with Class S_1S_2 male

PARENTAGE	PROGENY		TOTAL
	S_1S_2	S_2S_3	
22 *L1Q2*×*R1*	46	45	91
23 *L1Q2*×*R1*	40	36	76
39 *R1L1*×*R1*	28	20	48
Q1×*N1*	21	19	40
R3×*E2*	13	15	28
R3×*R1*	21	13	34
Total	169	148	317

Most probable expectancy 158.5 : 158.5. Deviation, 10.5 P. E. 6.

Altogether in these six experiments, 2,624 individuals were classified definitely by from one to fifteen pollinations made in various ways with test plants, without a single exception being found. In every cross the progeny fell into two groups of about equal size, and in no case was an individual produced belonging to the class represented by the mother. In addition, about 200 plants were grown which we were unable to classify precisely because unable to make the requisite pollinations. These plants are necessarily omitted from the records. They are reported as a matter of good faith; but it cannot be said that any one of them is an exception, because every plant given *all* of the necessary tests fell definitely into one or the other of the known classes.

Previously published data, recorded by Dr. E. S. ANDERSON, (1924) probably might have been added to the above results for the gr oups termed

X, Y and Z had we possessed the proper test plants to bridge the gap between his plants and our own. As things stand, all that can be said is that most of ANDERSON'S data are also concerned with three classes, and that our plants are descendants of the plants that he used. It is hardly to be questioned that our groups X, Y and Z are his groups A, B and C; but as to which is which we are not certain. Those of ANDERSON'S data that can be readily resolved into identical classes consistent with each other are as follows. Three crosses between A females and B males gave 25 B's and 22 C's. Three crosses between A females and C males produced 30 B's and 27 C's. One mating of C female with A male produced.7 A's and 9 B's. One mating between a B female and a C male gave 6 A's and 4 C's. And finally, the reciprocal of this cross, a mating of a C female with a B male, gave 4 A's and 6 B's. Thus in every case the group of the female parent was unrepresented, the progeny belonging to the other two groups, which were represented in approximately equal numbers.

A few other of ANDERSON'S figures come from the same family types, but the classes of the parents are unknown. In one case there was an apparent exception which was extremely puzzling. A family R (ANDERSON 1924, p. 32) consisted of 17 individuals belonging to class A and 20 individuals belonging to class C. Family X, which was supposed to have come from the reciprocal of the cross producing family R, gave results identical with those of family R. There were 10 individuals belonging to class A and 5 individuals belonging to class C. This situation was anomalous, for in no other case had reciprocal crosses produced plants belonging to the same two classes. Dr. ANDERSON luckily was able to solve the mystery because he had preserved the original seed envelopes. Several of the crosses used in his work had been made by an investigator who insisted on emphasizing male superiority by writing the male first in the laboratory records. Usually there was no confusion because the male and female signs were used, but in the particular case of family X, the seeming exception, these signs were left off though the figures were in the hand writing of the worker in question. According to the regular custom of this laboratory, therefore, family X was set down as the reciprocal of family R, when it actually was a duplicate of family R, and should have given the similar results that were actually obtained.

The behavior of plants carrying these three self-sterility allelomorphs, S_1, S_2 and S_3, was tested in other ways. Self-pollinations and incompatible cross-pollinations were rendered effective by pollinating buds about three days before they normally would open. Opportunity was thereby given for "like" gametes to fuse.

Family R1 was produced by selfing a plant belonging to class Y (S_1S_2). It consisted of 32 plants, of which 19 were typically class Y (S_1S_2), while 13 belonged to two new classes which were designated ZY and XY. Of the first class, ZY, there were 7 individuals. These plants were fertile as females with all classes except their own, but were sterile as males with classes Z (S_2S_3) and Y (S_1S_2) as well as with their own class. The 6 members of the second class, XY, were fertile as females with all classes except their own, but were sterile as males with class X (S_1S_3) and Y (S_1S_2) as well as with their own class.

Another class Y (S_1S_2) plant selfed, produced 39 individuals (Family Q2), of which 24 proved to belong to class Y, 14 to class ZY and 1 to class XY.

According to the selective pollen stimulation hypothesis class Y (S_1S_2) when selfed in the very young bud yields approximately 1 S_1S_1 (Class XY) + 2 S_1S_2 (Class Y) + 1 S_2S_2 (Class ZY). The S_1S_1 (XY) plants are fertile as females with

(a) Class Z (S_2S_3) because they accept both S_2 and S_3 pollen,
(b) Class X (S_1S_3) because they accept S_3 pollen, and
(c) Class Y (S_1S_2) because they accept S_2 pollen.

On the other hand, they are fertile as males with class Z (S_2S_3) because Z accepts S_1 pollen, but are sterile with both classes X (S_1S_3) and Y (S_1S_2) because neither of these classes accepts S_1 pollen.

Similarly individuals belonging to Class ZY (S_2S_2) are fertile as females with classes X, Y and Z, but are sterile as males with classes Y and Z.

Two other families were produced from this type of mating, but this time two different Y (S_1S_2) plants were crossed with each other reciprocally. The first cross (E2 × R1) yielded 17 class Y plants plus 5 class ZY plants plus 1 class XY plant. The reciprocal cross (R1 × E2) yielded 21 class Y plants plus 12 class ZY plants plus 6 class XY plants. Reciprocal crosses in this case, therefore, gave similar results as might be expected. The collected results from mating S_1S_2 plants together are found in table 7.

The data from these four families, each produced by mating S_1S_2 plants together, show clearly that there is a deficiency of S_1S_1 plants. There were 14 S_1S_1 plants, 81 S_1S_2 plants and 38 S_2S_2 plants. Apparently there is no marked selective fertilization, because there are just about one-half as many S_2S_2 plants as there are S_1S_2 plants. If heterozygotes were formed to the prejudice of homozygotes when there is equal opportunity for fertilization, it would seem as if there should be a great preponderance of S_1S_2 plants. This fact can be explained either by assuming that the S_2

pollen grows slightly faster than the S_1 pollen, or by S_1S_1 homozygotes being weaker than S_2S_2 homozygotes. Both of the homozygous classes are indeed weaker than the heterozygous class; but since they show practically the same vigor from time of germination to maturity, we are inclined to believe that the first postulate is correct. Briefly S_2 pollen grows slightly faster than S_1 pollen. Several additional critical crosses were made involving the homozygous class XY (S_1S_1), the results from which are strictly in accordance with hypothesis.

A homozygous S_1S_1 plant, 9 R1, was selfed in the young bud, giving us Family FA consisting of 31 plants. Each of these plants belonged to the class of the mother (S_1S_1).

TABLE 7

Results from mating Class Y (S_1S_2) plants together.

MATING	RESULT		
	S_1S_1	S_1S_2	S_2S_2
R1 (self)	6	19	7
Q2 (self)	1	24	14
E2×*R1*	1	17	5
R1×*E2*	6	21	12
Total	14	81	38

Second, two families were produced by crossing the same plant as above, 9 R1, a homozygous S_1S_1, with a plant O2 belonging to class S_1S_3. Family FB was the result of a normal pollination, Family FC was the result of a pollination made in the young bud. Since by hypothesis the female was S_1S_1 and the male S_1S_3, it is to be expected that only S_3 pollen would function in either case. That is to say, S_3 pollen sufficient to fertilize all the ovules would reach the ovary before any S_1 pollen would arrive. This is what happened. In Family FB the 24 plants tested all proved to belong to the one class S_1S_3, while in Family FC the 32 plants tested all proved to belong to the same class S_1S_3. Thus it seems that selective pollen-tube growth is a strictly individual reaction between the pollen and the style. The growth of a large group of compatible pollen-tubes does not appear to produce anything in the nature of a secretion which *materially* accelerates the growth of incompatible pollen-tubes.

On the other hand, either a *slight* acceleration to the growth of an incompatible pollen-tube may be furnished by the growth of a number of compatible pollen-tubes, or else pollen-tube growth in a *very* young bud may follow a different curve from that followed in the mature flower. For

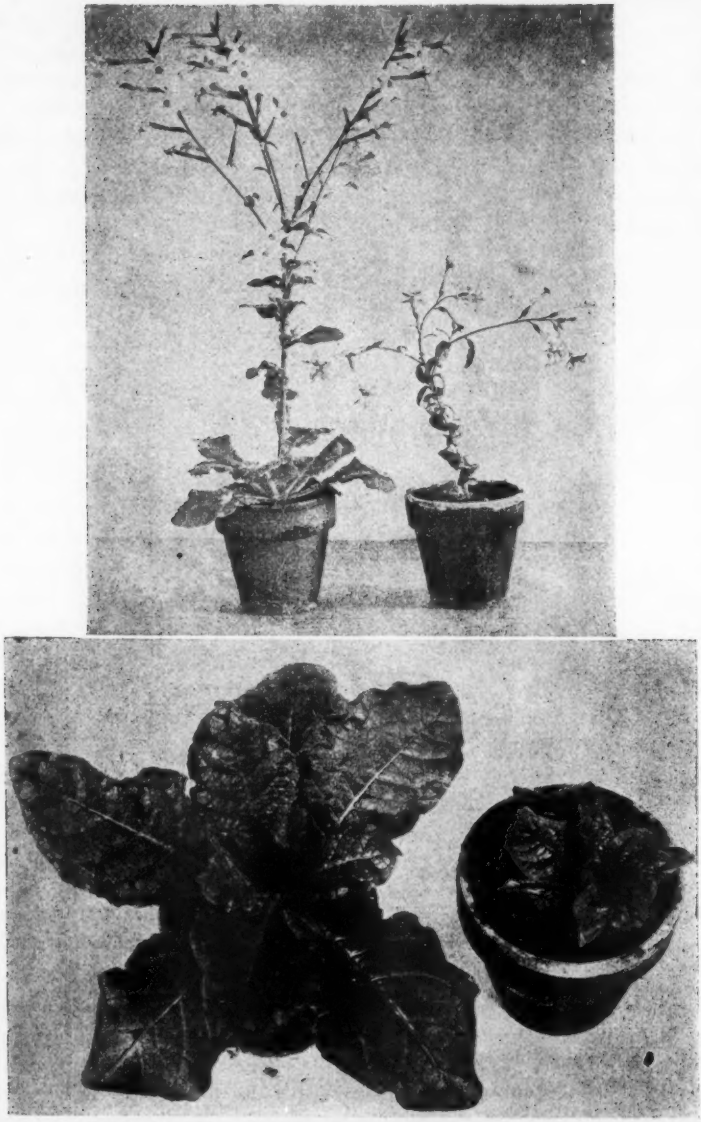

FIGURE 1. Plants of *Nicotiana Sanderae* Hort. showing the effect of the S_3 sterility factor in the homozygous condition. At left, S_1S_3 plants; at right, S_2S_3 plants.

example, normally such a mating as S_1S_2 female by S_1S_3 male gives only the two classes S_1S_3 and S_2S_3. But in one case such a cross made in the *very young* bud, the style being still very short, gave 13 S_1S_3 plus 13 S_2S_3 plus 4 S_1S_2 plus 4 S_1S_1. The same cross made on a mature flower gave 26 S_2S_3 plants and 14 S_1S_3 plants. That this type of phenomenon is not unique is shown further by the results obtained when a S_2S_3 plant (L1) was pollinated in the very young bud stage with pollen from a S_1S_3 plant (O2). The resulting population consisted of 21 S_1S_3 plus 15 S_1S_2 plus 11 S_2S_3 plus 13 S_3S_3. Since in this case an almost equal number of compatible and incompatible pollen-tubes reached the ovary, we are inclined to suggest that the growth-curves of the two types did not have the opportunity to show extreme differentiation because of the relatively short distance traversed. This point is being determined and will be reported upon later.

The final and most critical test of the hypothesis involving S_1S_1 plants was the pollination of two different flowers of the same individual, 9 R1 (S_1S_1), with pollen from plants belonging to classes Y (S_1S_2) and ZY (S_2S_2) respectively. Here are two different crosses, the first being class XY by class Y, the second being class XY by class ZY; yet by hypothesis only S_2 pollen should function in both cases, and all resulting plants should belong to class Y (S_1S_2).

Family FD consisted of 24 plants resulting from the mating 9 R1 (S_1S_1) by R1 (S_1S_2). All belong to class Y (S_1S_2). Family FF also consisted of 24 plants resulting from the mating 9 R1 (S_1S_1) by 19 Q2(S_2S_2). Again all belonged to class Y (S_1S_2).

Crosses involving the homozygous class ZY (S_2S_2) gave similar results. Plant R1E2 (S_2S_2) when selfed, produced 31 plants all belonging to the one group S_2S_2. Again this homozygous S_2S_2 plant, 4 R1E2, was crossed with pollen from R1 (S_1S_2) producing Family ED, and with pollen from 23 O2 (S_1S_1) producing Family EF. Cross $S_2S_2 \times S_1S_2$ yielded 24 plants all belonging to class S_1S_2, while cross $S_2S_2 \times S_1S_1$ also yielded 24 plants all belonging to class S_1S_2, as was expected.

A smaller series of tests were made with the heterozygous classes X (S_1S_3) and Z (S_2S_3) simply because the apparently lethal character of the homozygous class S_3S_3 diminished the opportunity for producing interesting combinations. But it now happens that the peculiarities of the S_3S_3 group makes it the most interesting of all. For this reason, the experiments with S_1S_3 plants and with S_2S_3 plants will be discussed together.

Two S_1S_3 plants were selfed. The first, O2, produced 30 plants of group S_1S_3 and 13 plants of group S_1S_1; the second, R3, produced 27 plants of

group S_1S_3 and 7 plants of group S_1S_1. Altogether 57 plants of class S_1S_3 and 20 plants of class S_1S_1 were obtained.

Likewise, when plant C13 (S_2S_3) was selfed, the resulting population consisted of 17 S_2S_3 plants and 12 S_2S_2 plants.

In none of these populations was a single S_3S_3 plant discovered; yet because there was no marked excess of heterozygotes over the types of homozygotes found, it seemed unlikely that the heterozygotes were produced wholly by the union of S_3 ovules and S_1 or S_2 pollen, as the case might be. It seemed more probable that S_3 pollen had reached the ovaries and had functioned in the production of heterozygotes. For this reason we concluded in our former paper (EAST and MANGELSDORF 1925) that S_3 is lethal when in the homozygous condition.

This conclusion is not wholly correct. In all three of these populations a number of abnormal seedlings appeared, apparently affected by root-rot. They were removed from the seed pans in order not to contaminate the healthy plants. Most of them died at an early age after showing typical root-rot symptoms. A few, however, grew slowly, came to maturity, and proved to belong to class S_3S_3. Every S_3S_3 plant found was of this type. The truth is, therefore, that each of these three populations yielded an unknown percentage of S_3S_3 plants.

While it is not strictly true that S_3 is lethal when in the homozygous condition, it certainly has lethal tendencies. During the winter months, with their short days, it is almost impossible to bring S_3S_3 plants to maturity. When grown during the spring and summer months they are somewhat more viable, but even then they seldom live long enough to produce more than a few flowers. The growth habit is peculiar to themselves. The leaves are more crinkled and the tips more rounded than their normal sisters, and each leaf has a characteristic outward roll, reminding one of spinach. Even fifteen-day-old seedlings can be separated unerringly by their appearance. The flowering branches of the older plants are usually weak and drooping. Efforts to keep plants alive long enough to mature seed capsules have thus far been unsuccessful. The flowers are normal, however, and the pollen in most cases is good. The plants can be classified, therefore, by their reactions as males.

The root-system is always reduced, as if by the action of root-rot. It is barely possible that the whole appearance is the result of a high susceptibility to root-rot, therefore; but the fact that most varieties of plants showing a root-rot infection sufficient to change their whole appearance do not live to maturity, makes it more probable that the morphological changes are due to the direct action of the factor S_3.

It is also possible, of course, that the type comes from the action of another hereditary factor closely linked to S_3, but thus far no crossovers have been found.

Among all of these tests, one can hardly imagine more critical experiments than those made on the homozygous families. Anyone who has puzzled his brain over the peculiar complications emerging from crosses between self-sterile plants will readily appreciate the weirdness of the following results. Here was a family of plants, XY, all inter-sterile. Another family of plants, ZY, was similarly inter-sterile. But XY individuals were fertile with ZY individuals; and reciprocal crosses for the first time produced similar results, for hitherto we had been dealing with matings between heterozygous types where the results of reciprocal matings were unlike. Every plant produced, either by cross XY × ZY or by cross ZY × XY, proved to belong to class Y. That is to say, these F_1 plants all belonged to a single sterility group, and this group was not the same as the two groups to which the parents belonged. Yet when tested as males on the F_1 plants, the two parental types, which had previously been shown to belong to separate classes, might readily have been classified together as one group. In other words, the parental classes XY and ZY were both sterile as males with all plants of their progeny, group Y. On the other hand, XY plants were fertile as females with pollen from Y plants and produced families of all Y plants. Likewise ZY plants were fertile as females with pollen from Y plants and again produced families of all Y plants,—a most astonishing result.

These seemingly anomalous and contradictory results are precisely what is to be expected by hypothesis. Class XY is S_1S_1, class ZY is S_2S_2. These two classes are fertile reciprocally and produce plants all of which belong to the one class Y = S_1S_2. Each of these S_1S_2 plants is sterile to the pollen of the parental classes; in the first case because S_1 pollen is not stimulated, and in the second case because S_2 pollen is not stimulated. On the other hand S_1S_1 plants are fertile with the pollen of S_1S_2 plants because S_2 pollen is stimulated and results in S_1S_2 plants being formed, while S_2S_2 plants are fertile with the pollen of S_1S_2 plants because S_1 pollen is stimulated and results in S_1S_2 plants being formed.

DISCUSSION

The analysis of the behavior of the self-sterile Nicotiana plants which has been made in this paper, does not complete even the factorial interpretation for Nicotiana. Another member of the same allelomorphic series, S_4, has been discovered and proved to be distinct from factors S_1, S_2 and S_3.

Three other factors have also been found in material from different sources which may prove to be different from those already described, but critical tests on them have not yet been completed. Nor is it certain that only one locus is involved in the control of self-sterility.

It is hoped that these matters can be more or less definitely settled within the next few months, and that some light can be thrown on the probable number and distribution of factors within these species. But even if matters should thus work out according to our most optimistic expectations, there is still much doubt as to the physiological meaning of the genetic results. It is clear that there is an interaction between the pollen-tubes and the tissues of the styles due to their genetic composition. But it is not certain whether *similar* genetic formulae cause inhibited growth, or whether *different* genetic formulae cause accelerated growth. This distinction may seem like a play on words, but there is a real difference which can be explained only when we know whether more than one locus is involved in controlling self-sterility and what are the exact types of the pollen-tube growth curves.

Some of these problems together with the question as to whether the interpretation of self- and cross-sterility here proposed can be extended to other groups of plants will be discussed in a later paper.

SUMMARY

1. In the populations of Nicotiana used in these studies the behavior and inheritance of self-sterility and of its corollary, cross-sterility, are shown to be determined by three allelomorphic sterility factors S_1, S_2 and S_3.

2. The action of these sterility factors is such that the growth of pollen tubes carrying a given factor is inhibited in the styles of plants carrying that factor.

3. As a result reciprocal crosses between plants having one of the sterility factors in common give unlike progenies, and the sterility group to which the female parent belongs is always absent in the progeny. S_1S_2 ♀ × S_1S_3 ♂ gives equal numbers of groups S_1S_3 and S_2S_3, while the reciprocal cross S_1S_3 ♀ × S_1S_2 ♂ gives equal numbers of groups S_1S_2 and S_2S_3. The other possible combinations give analogous results.

4. Populations produced by selfing in the young bud stage show the expected segregation. Group S_1S_2 selfed gives groups S_1S_1, S_1S_2 and S_2S_2. Group S_1S_3 selfed gives groups S_1S_1, S_1S_3 and S_3S_3. Group S_2S_3 selfed gives groups S_2S_2, S_2S_3 and S_3S_3.

5. Tests of various crosses between both heterozygous and homozygous plants have given the sterility and fertility reactions expected by hypothesis.

6. One of the allelomorphs (S_3) in the homozygous condition produces characteristic abnormalities in habit of growth.

7. New material now being tested has given evidence of additional sterility factors. Complete tests have been made on only one type. It proves to be a fourth allelomorph, S_4, belonging to the same series as those previously described.

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THE MATERNAL INHERITANCE OF A SEX-LIMITED LETHAL EFFECT IN *DROSOPHILA MELANOGASTER*

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SUMMARY

The present paper describes the maternal inheritance of a lethal effect in *Drosophila melanogaster* which leads to the early death of a majority of female zygotes, and which in consequence gives rise to an average sex-ratio among adults of one female to 5.5 males. The effect may be transmitted both through the male and through the female; the genetic basis involves a recessive gene in the second chromosome group. The missing females probably die in the egg stage. The maternal effect may be attributed to an influence of the chromosomal composition of the mother upon the eggs which will give rise to the offspring exhibiting the lethal ratio previous to the time when these eggs leave the mother's body. The presencein such eggs, after fertilization, of the female-determining chromosomal complex as contrasted with the presence of the male-determining chromosomal complex is responsible for the differential death rate.

[1] The data included under these two headings were obtained while the writer was Nationa Research Fellow in Zoology at COLUMBIA UNIVERSITY.

HISTORICAL

It has long been known, as shown for example by the extensive work on reciprocal species crosses in echinoderms and teleosts, that the developing embryo may for a longer or shorter period exhibit characters typical of the race from which the egg was derived. But an intrinsic difficulty from the genetic standpoint in much of this work is that the hybrids cannot be raised to maturity and bred. It is only when succeeding generations are obtained that we have the data necessary for determining whether a given case of maternal inheritance is quite independent of the nucleus or is the result of a deferred nuclear influence. The intimate relation of the egg with the mother's body, as well as the fact that preceding reduction it contains the chromosome complex of the mother, afford a basis for the latter type of maternal inheritance.

In regard to those characters which are shown to be determined by the genic composition of the mother and to which in consequence the term "maternal inheritance" is applied, it should be noted that this term is not happily chosen. For although the characters are immediately fixed by the constitution of the mother, they are just as definitely inherited from the father as from the mother, as becomes apparent in following generations.

The work of TOYAMA (1912 and 1913), TANAKA (1924), and others has demonstrated that cases of maternal inheritance shown by egg characters (shape, color, voltinism) of the silkworm moth may be explained on the basis of segregating genes. The color characters, which have been most extensively studied, depend upon the yolk (deposited while the egg is still part of the maternal body), the shell (derived from the epithelium of the oviduct), and the serosa. UDA (1923) has recently questioned the conclusions of TOYAMA regarding the transmission of brown versus slate, dependent upon serosa pigmentation; but it is clear from UDA'S paper, as has been pointed out by TANAKA (1924) and PELLEW (1925), that his data, as well as the discrepancies in TOYAMA'S papers, are readily explainable on the basis of an influence of the maternal genetic composition combined with an influence of the composition of the embryo itself.

STURTEVANT (1923) has suggested that the data of BOYCOTT and DIVER (1923) on the heredity of the direction of coiling in the snail, *Limnaea*, might be interpreted in terms of maternal inheritance. The recent paper of DIVER (1925) indicates that in addition to the parental nuclear influence the genetic composition of the zygote itself may be a further factor; there are possibly other as yet undetected influences at work in determining this character.

The studies of GOLDSCHMIDT (1924) on the transmission of the larval pattern resulting from crosses of geographic varieties of the gypsy moth, *Lymantria dispar*, have led him to conclude that besides the hereditary differences dependent upon segregating genes there is evidence of further differences which depend upon the plasma furnished by the egg and by the spermatozoon. In this respect, as is to be expected, the egg is the more influential. It may be recalled that GOLDSCHMIDT'S work on intersexes in this form shows that the "femaleness" factors are inherited, without paternal influence, exclusively from the mother. They were previously (1916 and 1917) attributed to the cytoplasm of the egg. It is now (1922 and 1923) suggested that they are transmitted by the "Y" or "W" chromosome and that a deferred effect is exhibited. This latter explanation is based upon the appearance of individuals assumed to be non-disjunctional.

Occasional characters are reported for *Drosophila melanogaster* whose expression shows more or less influence of the unreduced genetic composition of the mother. Such a maternal effect was reported by MORGAN (1912 and 1915) for rudimentary: by LYNCH (1919) in a study of a number of mutant forms showing partial or complete sterility in certain crosses; these forms include fused, rudimentary, morula, dwarf, and reduced bristles: and by MOHR (1925) for a third-chromosome recessive minute. An interesting point established by Miss LYNCH is the partial restorative effect upon viability at some stage of the presence of the normal allelomorph (for example of fused). This restorative effect may be exercised: (1) by the normal allelomorph of fused when present in the unreduced egg, even though the mature egg may contain fused and may be fertilized by a spermatozoon carrying fused, or (2) by the introduction of the normal allelomorph of fused by the spermatozoon.

Typical egg characters in *Drosophila melanogaster* are known to be determined by the genetic composition of the mother. MOHR (1922) found that the singed gene causes the eggs of homozygous females to be short and deformed. WARREN'S study (1924) of egg size in various races indicates that this character is dependent upon genes in all four linkage groups and that it is determined by the genetic constitution of the mother exclusively. This is to be expected since the size of the egg is fixed before the entrance of the spermatozoon. An interesting character of this type has recently been found by J. C. LI (unpublished work from the Columbia laboratory) in the eggs of Minute-l females. These eggs are shorter and rounder than normal eggs and are considerably more transparent so that developing structures are readily observed.

ACKNOWLEDGMENTS

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FIRST APPEARANCE OF THE LETHAL

The original culture exhibiting a sex-count with the low proportion of females appeared in February, 1923, among the progeny of a cross involved in an experiment designed to measure the rate of lethal mutation in the X-chromosome of *Drosophila melanogaster*. Three generations previously a female from wild type stock had been mated to a male from eosin-vermillion-forked stock. (Eosin, vermilion and forked are sex-linked recessives with the following symbols and loci: eosin, w^e, 1.5: vermilion, v, 33.0: forked, f, 56.5 BRIDGES, 1921). A phenotypically wild female from this cross, with one X-chromosome of the wild type, the other containing eosin, vermilion and forked, was mated to an eosin-vermilion-forked male. A similar selection was made among the progeny for the parents of the next generation; these were the parents of the lethal culture.

The expectation in such a cross is approximately equal numbers of males and females and approximately equal contrary classes. By the procedure of mating normal-appearing females with eosin-vermilion-forked sibs in successive generations, a new sex-linked lethal may be detected. For not only are there disturbances in the sex-ratio resulting from the death of males, but one class of each pair of male contrary classes is markedly smaller than the other. Thus a measure of the rate of lethal mutation in the X-chromosome may be obtained. (MULLER and ALTENBURG 1919).

TABLE 1

A. The original lethal culture, $\underline{w^e v f}$ ♀ × $w^e v f$ ♂. A deficiency in the male classes with the eosin end of the maternal $w^e v f$ chromosome is shown.

w^e		+		w^e		v		$w^e v$		f		$w^e f$		♀	
♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
0	8	1	44	0	10	0	19	0	4	0	9	0	0	0	2

The original culture gave, as table 1A shows, an unexpected count of one female and 96 males. Moreover, there seemed also to be discrepancies

in the male classes receiving the eosin end of the eosin-vermilion-forked chromosome of the mother. The one female was sterile, for she produced no offspring by either an eosin-vermilion-forked sib or by a fresh wild type male. Such sterility is frequent in the lethal strain. The explanation of the deficiencies shown by the male classes receiving the eosin end of the mother's eosin-vermilion-forked chromosome is not clear. They are probably not purely "fortuitous". That they are not due to the heterozygous presence in the mother of a sex-linked *semi*-lethal in this region is shown by the fact that four cultures, producing grandsons of the eosin-vermilion-forked males did not show such deficiencies. The totals show the following male contrary classes: 85 wild type, 60 $w^e vf$; 23w^e, 30vf; 29$w^e v$, 22f; 5$w^e f$, 6v; 262 females were produced. That the discrepancies in the eosin-containing male classes of the first lethal culture are not necessarily correlated with the production of the low female count is demonstrated by the following: (1) The lethal strain was derived from a non-crossover wild type male of this culture. (2) Later lethal counts were obtained from crosses of eosin-vermilion-forked males by females one of whose X-chromosomes contained eosin, vermilion and forked (the other X-chromosome was wild type), and these counts did not present comparable deficiencies in the male classes containing eosin. This is shown by table 1B.

B. Further crosses of $w^e vf$ ♀ × $w^e vf$ ♂ which give lethal counts. The lethal has been introduced from the lethal strain. No significant deficiency is shown in the male classes with the eosin end of the maternal $w^e vf$ chromosome.

	$w^e vf$	+	w^e	vf	$w^e v$	f	$w^e f$	v
	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂	♀ ♂
	8 25	3 35	1 17	2 18	1 5	1 8	0 2	0 0
	0 21	1 21	0 7	0 9	0 2	0 6	0 1	0 2
Total	8 46	4 56	1 24	2 27	1 7	1 14	0 3	0 2

The most plausible explanation of the irregularities in the contrary classes of table 1A involves the assumption that a new sex-linked lethal, quite a different lethal from that which kills females, arose in the mother of the culture. This new male-affecting lethal was supposedly located in the eosin end of the eosin-vermilion-forked chromosome and was not present in all of the unreduced eggs. The latter would necessarily be true since those of the surviving eosin-vermilion-forked sons which were tested did not transmit such a lethal. Sex-linked lethals of this type are ordinarily kept by breeding the heterozygous females. The procedure was impossible in this case because these females were killed by the maternally inherited

lethal. That is to say, the only individuals which would be expected to survive and show evidence of such a sex-linked lethal were killed by another lethal. It is obvious that this hypothesis remains tentative, since of necessity it is not amenable to test.

It should be noted concerning the cultures of table 1B that the lethal counts given are of F_2 offspring of a cross of an eosin-vermilion-forked female from the mutation rate experiment mentioned above, by a lethal male. The grandmother consequently is from the strain from which the female-killing lethal originally was derived. The interpretation of maternal inheritance employed later in the paper implies that if out-crosses are made of lethal males to non-lethal strains and the progeny are inbred, the female-deficient counts should not be produced until the F_3 individuals are obtained. However, if both parents should contribute the lethal, such counts might appear before F_3. Since the grandmother of the individuals included in table 1B was from the strain from which the lethal was derived, it is reasonable to assume that she may have been heterozygous for one or more genes concerned. If so, these cultures show no unexpected results. Of the 256 cultures which exhibit the lethal count (included in figure 1) these two alone show characteristics at first sight (and only at first sight) not compatible with the interpretation of maternal inheritance.

The exact conditions under which the lethal originally arose are unknown. Judging by its subsequent behavior, which shows that the mother of a lethal count must be homozygous for a second chromosome recessive, it seems most probable that this recessive was already present in the eosin-vermilion-forked stock which appears in the pedigree of the strain. If so, the several intervening generations produced the genetic combination conducive to the appearance of the female-deficient ratio.

CHARACTERISTICS OF THE LETHAL

The most outstanding feature of the lethal is, of course, the low proportion of females produced. The total number of flies from all cultures known to exhibit the lethal ratio gives 3,982 females and 21,821 males; thus the average sex-ratio is 1 female : 5.5 males. However there is a great variation in the sex-ratio produced by different cultures; for although a considerable proportion of the cultures produce very few females, or none, in others the sex-ratio more nearly approaches equality. This variation is shown by figure 1.

It should be stated concerning the cultures included in figure 1 that there has been an appreciable increase since the discovery of the mutant in the number of females produced per bottle. Of the cultures producing no

daughters, a relatively larger proportion appeared early in the work; the large number of cultures giving 20 to 30 percent females came late. Since the environment was the same at these two periods the effect may be attributed to selection. It is obvious that the strain would be perpetuated by matings from those bottles which, although showing definite lethal ratios, gave female offspring. Modifiers would thus be selected for whose tendency would be partially to counteract the lethal effect.

A few cultures producing sex-ratios intermediate between 1 female : 2 males and 1 female : 1 male were excluded from the totals and from figure 1 because, although several of these were shown by breeding tests to contain the lethal (there were about 6 of these; they would not substantially alter the sex-ratio of 1 female : 5.5 males), others were not so tested, and some of these latter undoubtedly did not represent lethal counts. Experience with non-lethal lines demonstrates that occasional fortuitous ratios of this type

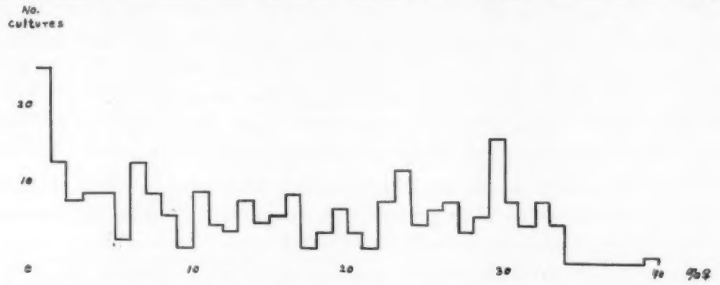


FIGURE 1. Variation in the female percentage of the total number of flies in cultures exhibiting the lethal count.

are produced, particularly when the number of offspring is not large. The tendency of such of these cultures as may have been lethal slightly to alter the sex-ratio would, moreover, be at least largely offset by the inclusion of cultures undoubtedly representing lethal counts which were excluded on the basis of their very low production.

Especially unfortunate attributes of the lethal strain are its high sterility and its low productivity. Large numbers of individuals produce no offspring whatever, and this seems to be due to sterility in both sexes. When offspring are produced, the numbers frequently are very small. Broods are produced, however, as an examination of the tables shows, which are large in size. The factors of low productivity and of high sterility have made the collection of data difficult. Indeed it is probable it would not have been considered worth while to preserve the strain, had it not been for a peculiarity of behavior indicating maternal inheritance.

Other characters of the lethal strain include various morphological abnormalities. Chief among these is the presence in all lethal cultures, of broken abdominal bands (at least since this character was discovered very early in the work). There is considerable variation in the extent to which this character is exhibited. Most, if not all, broken bands seem to be dependent upon a tendency to reduction in the dorso-lateral plates; in some individuals one of these plates is completely suppressed. Probably at least some of the sterility of the strain is correlated with these broken bands, for frequently the external genitalia of broken banded individuals are abnormal. The ratio of broken to non-broken is not appreciably different in the two sexes. The broken bands are found not only in bottles exhibiting the lethal count; they are also found in cultures derived from the lethal strain which do not show a lethal ratio. Individuals known to be heterozygous for the second chromosome recessive involved may have broken bands. The exact relation of the broken bands to this recessive lethal gene is not known; they may be dependent upon other genes. The matter is probably complicated by an environmental effect.

Other abnormalities occurring in the lethal strain with many times the frequency shown by normal stocks include: (1) a slight roughening of the eye, (2) a stunting of one wing or its complete absence; this is usually accompanied by a deformity of the thorax on the same side, (3) a reduction in the size of one or both eyes, and (4) a shrivelling or absence of one leg. Recently these characters, excepting the first, have largely disappeared. It seems probable that they were not definitely correlated with the presence of the lethal gene, but were independent characters present in masked condition in the stock and were brought out by the intensive inbreeding which was practised. It may be noted, however, that abnormalities of this type are common in individuals in which the developmental process is in any way radically upset.

Departures from the normal sex-ratio in *Drosophila melanogaster* are commonly due to an elimination of males by lethal or semi-lethal genes in the X-chromosome. However, sex-ratios exhibiting a deficiency in the number of females produced, although rare, have previously been reported. Subjection to a temperature of 31.5°C. during pupation has been found (MANN 1923) slightly to increase the number of males. Moreover, mutant characters are known, as truncate (ALTENBURG and MULLER 1920), which are somatically somewhat more pronounced in the female than in the male, and which in consequence lead to a somewhat lower relative viability in females. A sex-limited, sex-linked lethal was reported by THOMPSON (abstract, 1921) at the 1920 meeting of the AMERICAN SOCIETY of ZOO-

LOGISTS. This lethal killed all females homozygous for it; males with the lethal were perfectly viable, but held the wings erect. Crosses of heterozygous females with lethal males gave sex-ratios of 1 female : 2 males.

A similar lethal has more recently been described by BONNIER (1923). This lethal kills homozygous females, but has no apparent effect on the males containing it. As in the above case, it gives sex-ratios of 1 female : 2 males. BONNIER has shown how by its means sex-dimorphism can be simulated. He also predicts the finding of autosomal lethals which kill one or the other sex. This prediction is verified by the present lethal.

There is recorded in a recent paper on attached X-chromosomes (L. V. MORGAN 1925) a single culture (No. 184) producing a relatively large number of males. This is from a strain of peculiar behavior (being investigated by T. H. MORGAN) in which the ratio of males to females is variable but high.

Of a different type were the sex-ratios of the unisexual broods reported in 1910 by QUACKENBUSH. One of these cultures produced 135 males and no females, a ratio much like those given by the lethal now reported. It seems reasonable, as has been suggested by STURTEVANT (1920), that the sex-ratios obtained by QUACKENBUSH were due to crosses between *Drosophila simulans* and *Drosophila melanogaster*, which two species were at that time not distinguished (STURTEVANT 1919). The derivation of the parents from mass cultures is suggestive. The sterility of the flies constituting these unisexual broods and their rudimentary gonads, as well as the sex-ratios produced, are all characteristic of crosses of these species, as STURTEVANT'S work shows. In regard to the culture mentioned above which gave a count showing no females, it may be stated that the cross of *Drosophila simulans* female by *Drosophila melanogaster* male, but not the reciprocal cross, gives a greatly lowered proportion of females; the reciprocal cross gives all females.

Striking similarities are shown between a lethal in *Drosophila funebris* reported by MOHR and STURTEVANT (1919) and the present lethal. These investigators found a brood consisting of one female and 87 males. Sex-ratios showing the excess of males were repeated in later generations, but as in the *melanogaster* lethal, there was considerable variation in the sex-ratio, for there was a range from cultures producing no females to cultures in which the sexes were approximately equal in number. Broken abdominal bands were present, but unlike those occurring in *melanogaster* were only rarely found in males. It was believed that those individuals with extreme broken bands did not emerge from the pupa cases. When the strain was crossed to unrelated races the F_1 sex-ratio was 1 female : 1 male, and

broken abdominal bands did not occur. Both characters reappeared in F_2. This lethal does not, therefore, show maternal inheritance, and in this respect also it is different from the *melanogaster* mutant.

PROOF OF THE MATERNAL INHERITANCE OF THE LETHAL

In demonstrating the maternal inheritance of the lethal it will be shown that the reciprocal out-crosses produce different results. This, however, is true in out-crosses of ordinary sex-linked mutants. The further demonstration is then necessary that the character reappears for the first time in F_3 rather than in F_2. It will be shown also that the presence in the mother in homozygous condition of a second chromosome gene is necessary in order that the offspring exhibit the lethal count.

It may be stated at the outset that this second chromosome recessive is apparently not the only necessary element involved. This is indicated particularly by the fact that individuals of the same genetic composition with regard to this gene do not always give the same breeding results. That is, cultures sometimes do not give lethal counts which are expected to do so. The converse is not true. The accessory factors are not known; it is possible that environment plays a role.[1] There are indications that modifying or complementary genes exist. This statement is based upon: (1) the relative difficulty with which lethal counts are again obtained once the strain is out-crossed, even when the second chromosome is traced, and (2) the fact that a given female once having given a lethal ratio continues to do so when she is transferred to new bottles. This is strikingly brought out in the work on determining the time of death of the daughters, when it was necessary to make daily transfers of the parents. It has not been considered worth while to attempt a genetic analysis of these modifying genes. Their presence in no way invalidates the conclusion that the lethal effect is maternally inherited and is dependent upon a second-chromosome recessive.

Out-crosses of lethal females

Table 2 shows the immediate results of crosses of females from the lethal strain with males of the stocks indicated. The following genes, in addition to those previously enumerated, are involved. In chromosome II are: star, *S*, 0.0; black, *b*, 46.5; purple, p_r, 52.5; curved, *c*, 73.5 (BRIDGES and MORGAN 1919 and BRIDGES 1921). The curly used is a second chromosome

[1] Rather extensive attempts to vary the environment by means of acid and temperature have not yielded significant results, chiefly because of the difficulty of breeding this strain under conditions not optimum.

TABLE 2

Crosses exhibiting the lethal count; the female parent in each case is from the lethal strain, the male parent of the type indicated is of different stock.

A. The female parent is known to be virgin.

	P₁ ♂	♀	♂	♂ per 1 ♀
	w^a v f	16	110	6.9
	w^a v f	1	69	69.0
	b pr c	17	98	5.8
	b pr c	1	126	126.0
	b pr c	11	71	6.5
	b pr c	26	106	4.1
	b pr c	1	121	121.0
	b pr c	16	126	7.9
	b pr c	36	128	3.6
	b pr c	0	49	. .
	b pr c	9	46	5.1
	b pr c	0	35	. .
	b pr c	38	105	2.8
	b pr c	3	39	13.0
	b pr c	10	53	5.3
	b pr c	1	124	124.0
	b pr c	8	129	16.1
	b pr c	22	50	2.3
	b pr c	6	96	16.0
	b pr c	0	83	. .
	b pr c	38	164	4.3
	b pr c	2	162	81.0
	IIIple	18	48	2.7
Total		280	2138	7.6

*B. The female parent is not known to be virgin; for cultures marked * she is known not to be virgin.*

				SHOWING DOMINANT CHARACTERS		
	P₁ ♂	♀	♂	♀	♂	♂ per 1 ♀
	S D	2	57	0	28	28.5
	S D	0	90	0	63	. .
	**S D*	18	158	7	82	8.8
	**S D*	38	166	2	13	4.4
	S D	27	65	4	38	2.4
	S D	5	44	1	30	8.8
	Curly	1	56	1	27	56.0
	Curly	5	83	1	14	16.6
Total		96	719	16	295	7.5

stock of the composition $C_{CL}C_{y}c_{n}^{2}C_{CR}/+$. C_{y} is the gene for curly wings which is generally lethal in homozygous condition, c_{n}^{2} is an allelomorph of cinnabar, and C_{CL} and C_{CR} prevent crossing over (WARD 1923). In this paper "curly" and "non-curly" offspring of the cross of curly stock by the lethal will be mentioned; the former designates offspring containing the C_{y}-chromosome of the curly stock, the latter may or may not (as will be made clear in the proper place) designate offspring containing the other chromosome. Dichaete, *D*, 40.0, is a third chromosome mutant. The IIIple stock used includes the following third chromosome recessives: roughoid, r_{u}, 0.0; hairy, h, 26.5; scarlet, s_{t}, 43.8; peach, p^{p}, 48.0; spineless, s_{s}, 58.5; sooty, e^{s}, 70.7 (BRIDGES and MORGAN 1923). It may be further noted that star and dichaete are both dominants and are both lethal when homozygous.

The cross of a female from the lethal strain by an unrelated male may, then, produce the lethal count, and this is true of all the stocks from which males were adequately tested. The lethal counts are not obtained among the F_2 progeny, but may reappear in F_3. The cultures included in table 2B are of particular interest. The female parent of the cultures starred was in each case purposely crossed first to a sib and later to a star-dichaete male. The fact that considerably less than three-fourths of the offspring exhibit one or both of the dominant characters indicates that the female produced offspring by both males. The same is undoubtedly true of the female parent of the last culture (in which cross the expectation is somewhat more curly than non-curly due to the greater relative viability of curly), although she was not intentionally double-mated. In these cases the lethal count is exhibited by the offspring showing dominant characters; moreover after the subtraction of the expected number of normal offspring (assuming that she was mated with the dominant male alone) from the actual number of normal offspring, the remainder also exhibits the lethal count. These crosses show, therefore, that not only may a female from the lethal strain produce a lethal count when mated to a male of unrelated stock, but a single female may be crossed in succession to two different types of male, and the lethal count be shown among the offspring of both males. This is to be expected in the case of a maternally inherited character.

Out-crosses of lethal males

Table 3 shows the results of the cross of males from the lethal strain with females from unrelated stocks. The sex-ratio in F_1 is 1 : 1. The same ratio is shown by the offspring of inbred F_1; it was thought unnecessary

to tabulate these results. In the F_3 obtained from these F_2 the lethal counts reappear, as the table shows.

TABLE 3

Crosses not exhibiting the lethal count; the female parent in each case is from the stock indicated, the male parent is from the lethal strain. As shown, offspring of these cultures exhibit the lethal count in the third generation.

	P_1 ♀	♀	♂	♂ per 1 ♀	F_1 lethal counts		
					♀	♂	♂ per 1 ♀
	S D	64	64	1.0	1	84	84.0
	S D	17	18	1.1	0	42	..
	S D	34	38	1.1	0	75	..
		..	..	..	2	54	27.0
	S D	60	52	.9	3	42	14.0
	Curly	49	50	1.0	0	146	..
		..	..	..	8	113	14.1
	Curly	10	11	1.1	11	152	13.8
		..	..	..	0	131	..
		..	..	..	0	106	..
	Wild-type	110	104	.9	2	98	49.0
		..	..	..	11	51	4.6
	Curly	64	49	.8	26	90	3.5
		..	..	..	0	106	..
	b pr c	111	117	1.1	17	98	5.8
		..	..	..	1	126	126.0
		..	..	..	11	71	6.5
		..	..	..	26	106	4.1
		..	..	..	1	121	121.0
		..	..	..	16	126	7.9
		..	..	..	36	128	3.6
		..	..	..	0	49	..
		..	..	..	9	46	5.1
		..	..	..	0	35	..
		..	..	..	38	105	2.8
		..	..	..	10	53	5.3
		..	..	..	1	124	124.0
		..	..	..	8	129	16.1
		..	..	..	22	50	2.3
		..	..	..	6	96	16.0
		..	..	..	2	162	81.0
	b pr c	21	28	1.3	3	39	13.0
	IIIple	49	55	1.1	18	48	2.7
	IIIple	47	36	.8	0	83	..
	$\frac{S \quad D}{\text{Curly} \quad +}$	..	..	...	38	164	4.3
		39	37	.9	1	28	28.0
Total		675	659	1—	Total 327	3327	10.2

Reciprocal crosses, then, produce entirely different results in F_1. The disappearance of the lethal ratio in the cross of lethal male by unrelated female is purely temporary, since the peculiarity reappears in F_2.

Crosses from the curly-lethal strain

It was found in crosses between flies from the lethal cultures and flies from curly stock, that when the progeny were inbred, lethal counts might be obtained from non-curly females but they were never obtained from curly females. This is illustrated by tables 4 and 5. The parents of the two sets of cultures shown here differ, so far as known, only as regards one chromosome. For the male parents of both sets are curly and are from the inbred curly-lethal strain; the female parents of table 4 are non-curly

TABLE 4

Crosses exhibiting the lethal count; the parents are from the strain derived by crossing curly stock with the lethal. The female parent is non-curly; the male parent curly.

	♀	♂	♂ per 1 ♀
	0	131	..
	15	64	4.3
	4	50	12.5
	6	54	9.0
	24	74	3.1
	1	96	96.0
	20	133	6.7
	2	76	38.0
	6	67	11.2
	27	58	2.2
	7	127	18.1
Total	112	930	8.3

and from the curly-lethal strain, the female parents of table 5 are curly from this strain. The former females produce lethal counts, the latter do not. The latter are heterozygous for the second chromosome received from the lethal stock. The former, it is assumed, have become homozygous for this chromosome. Corresponding to the fact that not all non-curly individuals would be expected to be homozygous for this chromosome (since some of them must contain the homologue of the chromosome containing curly in the curly stock), is the fact that not all such non-curly females give the lethal count. That the curly female parents of the cultures of table 5 contained the lethal, although they did not show it, is demonstrated by the fact that their non-curly daughters again produced the lethal counts, as shown. In other words, to produce the lethal count, a

female must be homozygous for the second chromosome received from the lethal strain; that is, she must be homozygous for a second chromosome recessive.

TABLE 5

Crosses not giving lethal counts; both parents are curly and are from the strain derived by crossing curly stock with the lethal. From the non-curly daughters lethal counts were again obtained, as indicated.

				Lethal counts from F_1 non-curly ♀♀			
	♀	♂	♂ per 1 ♀		♀	♂	♂ per 1 ♀
	84	50	.6		11	152	13.8
	..	..	..		0	131	....
	..	..	..		0	106	....
	91	75	.8		0	146	....
	88	87	1—		26	90	3.5
	..	..	.		0	106	....
	74	70	.9		39	103	2.6
	..	..	...		30	67	2.2
	105	99	.9		24	83	3.5
	96	102	1.1		12	63	5.3
	..	..	..		32	91	2.8
	52	62	1.2		9	59	6.6
	58	52	.9		1	96	96.0
	47	60	1.3		21	164	7.8
	43	29	.7		37	113	3.1
	..	..	..		21	103	4.9
	145	162	1.1		10	60	6.0
	119	130	1.1		6	67	11.2
	109	112	1.0		27	58	2.2
	76	74	1—		28	59	2.1
	..	..	..		20	52	2.6
	101	86	.9		2	36	18.0
Total	1288	1250	1—	Total	356	1905	5.4

Figure 2 represents a typical pedigree chosen to illustrate the genetic behavior of the lethal. To avoid irrelevant complexity only cultures are included which are in the line of ancestry of lethal counts. As is shown, a lethal male was mated to a female from curly stock, and this cross produced the offspring of culture A which showed a 1 : 1 sex-ratio. Two lines were established from this culture, in one the curly offspring of A were chosen as parents, in the other the non-curly offspring. The mating of curly individuals, B, gave a sex-ratio as expected of 1 female : 1 male. It is to be noted that the only non-curly chromosomes of the second pair contained in this line were necessarily from the lethal strain. Non-curly individuals

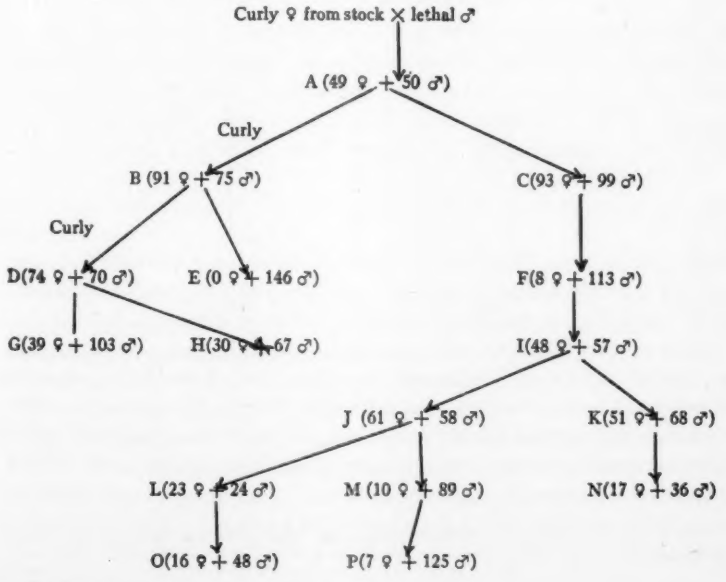
would be expected to produce lethal cultures. This expectation is verified by the cultures E, G and H.

The second line established, which was from the non-curly offspring of A, arose from the culture C. The parents of this latter culture are heterozygous and are expected not to give a lethal count; C shows a 1 : 1 sex-ratio. But the culture established from offspring of C might involve a mother homozygous for the lethal, correspondingly F does show the lethal count (these are the F_3 of the original cross). Not all of the females of this strain would be expected to give lethal counts, and they do not. All of these results are explicable on the basis of the presence of a second chromosome recessive. All other pedigrees involving the lethal may be interpreted on this same basis, although some of the results indicate the influence of modifying genes, as has been previously explained.

Since it seems unlikely on the basis of the breeding difficulties involved that the mutant will be of particular value in future experiments, the exact locus of the recessive gene in the second linkage group has not been determined. Incomplete data suggest a position near purple (52.5).

FIGURE 2

A typical pedigree showing the genetic behavior of the lethal.



CROSSES WITH A STRAIN WITH ATTACHED X-CHROMOSOMES

Whether the Y of the male, whose sisters die, is responsible *per se* for his survival is a question of interest. If this were true, females containing one extra chromosome, a Y, should be quite as viable as their brothers. To investigate the problem double yellow was used; both sexes of this strain contain a Y chromosome. The X-chromosomes of the female are more or less permanently attached (L. V. MORGAN 1922, 1925) and both contain the recessive gene yellow (*y*, 0.0). A female of this stock, then, obtains her X-chromosomes from her mother alone, she receives also a Y from her father. The male, on the other hand, receives his X from his father and his Y from his mother. It is to be expected that if the lethal is crossed to this double yellow stock, and if the presence of a Y chromosome is responsible for the survival of flies containing it, then succeeding generations should never yield lethal sex-ratios. But in such crosses lethal ratios (accompanied by broken abdominal bands) were obtained, as is shown by table 6; and in the expected proportion of cultures.

TABLE 6

Crosses giving the lethal count; the female parent of each is double yellow.

	Super ♀	♀	♂	♂ per 1♀
	0	43	87	2.0
	0	28	61	2.2
	0	27	67	2.5
	1	31	67	2.2
	0	37	75	2.0
	0	20	50	2.5
	0	43	89	2.1
	1	37	79	2.1
	0	26	55	2.1
	0	4	45	11.3
	0	0	77	....
Total	2	296	752	2.5

These results, however, are possibly not to be regarded as significant, for it was learned that a lethal somewhat similar in behavior is present in the attached-X stocks and sporadically gives female deficient ratios. This lethal is being studied by T. H. MORGAN. The lethal counts shown in table 6 are not, therefore, known with certainty to be due to the second-chromosome maternally inherited lethal.[1] Hence they do not prove, as

[1] Data from the crosses with double yellow have not been included in figures or tables other than table 6.

might offhand be concluded, that the Y is not responsible for the greater male viability. The question still remains open.

These crosses with the double yellow stock have bearing, however, on another problem. It might conceivably be held (although in view of considerations later to be presented, this is illogical) that the fact that the female zygotes do not appear is due to their never having been formed. Such an interpretation implies a gametic incompatibility in the lethal strain between an egg from a homozygous mother and the sperm which carries *X*. Now in the strain derived from the cross of lethal by double yellow, the sex resulting from such a combination is male (instead of female as is normally the case). If gametic incompatibility holds, therefore, these crosses will yield male-deficient sex-ratios rather than the female-deficient ratios present in the pure lethal strain. This is not the case; in the crosses involving double yellow no such reversed sex-ratio was obtained. It is believed that this fact probably cannot be attributed in each case to the exact counteracting (or overbalancing) effect of the presence of MORGAN'S lethal. It may be stated, therefore, that this evidence indicates that gametic incompatibility is not responsible for the female-deficient sex-ratio. The conclusion is strengthened by further evidence, as will be shown.

TIME OF DEATH OF FEMALES

On the assumption that gametic incompatibility is responsible for the abnormal sex-ratios it would be expected in the pure lethal strain that practically all eggs from homozygous mothers would be fertilized by Y-containing sperm, since X-containing sperm would be relatively impotent as regards fertilization. The number of males resulting, therefore, would be twice as large as in cultures from the same strain raised under similar conditions and showing non-lethal counts. This apparently is not true.

The fact that the number of offspring in lethal cultures is on the whole approximately half that of non-lethal cultures of the same strain, further shows that the females have not been "transformed" into males. The absence of intersexes tends also to preclude this possibility.

The above reasoning leads to the conclusion, therefore, that the female zygotes are formed but die. Dissection of pupae about ready to hatch shows lethal sex-ratios; death has consequently occurred before this stage.

In order to determine exactly when these zygotes die females were isolated and transferred daily. Each vial in which the females were kept was provided (according to the method of J. C. LI) with a narrow glass slide upon which was placed a slightly smaller strip of dark colored

blotting paper impregnated with fermented banana. Eggs were laid on the banana and showed up clearly against this background. The eggs were counted daily; after the elapse of several days the strip was again examined and the number of empty egg cases counted; this number is equivalent to the number of larvae which hatched (empty egg cases are more readily counted than burrowing larvae); a few days later the pupae were counted; as were the adults which emerged.

The conclusions which follow are based upon observations of the development of over 3,500 eggs from females producing lethal counts and of over 2,000 control eggs from a normal strain. Lethal females laid quite as many eggs as the control females. A marked discrepancy occurs, however, in the number of eggs which hatch—only 48 percent of the lethal eggs hatched as opposed to 73 percent of the normal eggs (this percentage is said to be low for normal eggs). Of the larvae from the lethal strain 70 percent pupate, of the larvae from the normal strain 86 percent. Of the pupae from the lethal strain 85 percent hatch into adults; of the normal pupae 95 percent. Thus it is obvious that although the embryo of the lethal strain has a lower viability throughout the entire developmental process, the difference in death rate is much more pronounced in the egg stage than at any other period. Although it cannot be stated definitely that all the females of the lethal strain which do not survive die as eggs, it seems probable that a large majority of these females die at that stage.

DISCUSSION

If a female from the lethal line is crossed to a male from an unrelated stock, the offspring immediately resulting may exhibit the lethal sex-ratio. But if the reciprocal cross is made, the F_1 never exhibit this ratio. Both crosses, alike, give lethal counts in F_2. The immediate offspring of the cross presumably, then, have the same genetic composition (whatever modifiers exist cannot be sex-linked); the only difference being that when the lethal is introduced from the mother most of the daughters die, but when it is introduced from the father they live. In other words, whether a given daughter lives or dies depends not upon her own genetic composition, aside from the fact of her being a female, but upon that of her mother.

We may draw further inferences regarding the nature of this maternal inheritance. It is obvious that the greatest difference between the female zygotes of the reciprocal crosses lies in the origin of their cytoplasm and shell. When the cytoplasm and shell are derived from the lethal line most of the daughters die, but when they are derived from the non-lethal line the daughters live. The cytoplasm or shell (or both) of the eggs from fe-

males of the lethal strain has (or have) been so affected that the combination with two X-chromosomes and the autosomes is very much less viable than the combination of non-lethal cytoplasm plus shell with two X-chromosomes and the autosomes, or than lethal (or non-lethal) cytoplasm and shell with X plus Y plus the autosomes. In this connection the reciprocal species crosses of *Drosophila simulans* with *Drosophila melanogaster* (STURTEVANT 1920) may be recalled. As with the present lethal the female offspring of these crosses are rarely able to develop if the egg cytoplasm and shell have been derived from one of the lines, but develop readily if they have been supplied by the other line. That the influence in the case of the lethal now reported is dependent in the final analysis upon the nucleus rather than upon independent cytoplasmic materials is shown by the fact that it is inherited as a second chromosome recessive.

If, in the lethal strain, the chromosomes of a given egg are assumed to be directly responsible for the effect on that egg, it seems probable that the effect becomes established previous to maturation, since, although half of the reduced eggs of a heterozygous female contain the second chromosome recessive, they do not show the lethal effect. However it is not necessarily the case that the chromosomes of the egg itself are responsible for the influence—the effect might conceivably result indirectly from the activity of the maternal chromosomes by means of substances circulating in the blood, or by absorbed materials from the interstitial cells of the ovary, or by means of the egg shell, or by other means. In case the influence is of this indirect type, it may or may not be impressed upon the egg after reduction.

CONCLUSIONS

1. There has been found in *Drosophila melanogaster* a lethal effect the appearance of which is governed by the genetic composition of the mother.

2. The sex-ratio of cultures exhibiting the lethal ratio is variable; the average sex-ratio is 1 female : 5.5 males.

3. A female whose progeny show the lethal sex-ratio must be homozygous for a second chromosome recessive gene.

4. The death of the females which do not appear as adults, probably occurs chiefly in the egg stage. It may be attributed to an influence of the maternal genetic composition upon the eggs which will give rise to the offspring exhibiting the lethal ratio, previous to the time when these eggs leave the mother's body. The presence in such eggs of the female-determining chromosomal complex results in a much higher death rate during development than does the presence of the male-determining chromosomal complex.

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